AD_____

Award Number: W81XWH-07-1-0259

TITLE: Exploiting the Immunological Effects of Standard Treatments in Prostate Cancer

PRINCIPAL INVESTIGATOR: Brad H. Nelson, Ph.D.

CONTRACTING ORGANIZATION: British Columbia Cancer Agency
Victoria BC V8R 6V5
Canada

REPORT DATE: March 2009

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

X Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
March 31, 2009	Annual Report	1 MAR 2008 - 28 FEB 2009

4. TITLE AND SUBTITLE
Exploiting the immunological effects of standard treatments in prostate cancer

5a. CONTRACT NUMBER
W81XWH-07-1-0259

5b. GRANT NUMBER
PC060159

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Brad H. Nelson, Ph.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

British Columbia Cancer
Agency,
2410 Lee Avenue,
Victoria BC V8R 6V5
Canada

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
US Army Medical Research and
Material Command
Fort Detrick, Maryland
21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT We previously demonstrated that hormone therapy (HT) and radiation therapy (RT) induce tumor-specific autoantibody responses in human prostate cancer, and this grant investigates the clinical significance of these findings. In Aim 1, the findings that HT induces autoantibody and T cell responses against PABPN1 in the Shionogi tumor model and that these immune responses are associated with inferior outcomes have recently been submitted for publication. We have also shown that the combination of HT+RT in this model leads to delayed tumor recurrence of a distal untreated tumor. Work is underway to determine whether similar antibody and T cell responses are seen in these mice and whether they too are associated with poor outcomes. In the human setting, we have tested known prostate cancer tumor antigens by ELISPOT and begun cloning our serologically-defined tumor antigens in order to test these against PBMCs collected from prostate cancer patients showing treatment-induced autoantibody responses (Aim 2). We have also continued to assemble cohorts of prostate cancer patients with recurrent versus non-recurrent disease at 5 years post-treatment (Aim 3). In summary, this study is progressing on schedule and is revealing unexpected results that we believe may be highly relevant to prognosis and treatment of prostate cancer.

15. SUBJECT TERMS
Tumor immunology, immunotherapy, prostate cancer, antibody, T cell, tumor antigen, hormone therapy, radiation therapy

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	40	USAMRMC

Table of Contents

W81XWH-07-1-0259 Annual Report, March 2009

PI: Brad H. Nelson, Ph.D.

Title of Project: Exploiting the Immunological Effects of Standard Treatments in Prostate Cancer

INTRODUCTION:

While much effort is being made to develop effective immune-based therapies for prostate cancer, there is little information available on whether standard treatments induce tumor-specific immune responses, which could potentially influence clinical outcomes. Radiation therapy causes inflammation associated with the expression of inflammatory cytokines, MHC molecules, B7 and other co-stimulatory molecules. Likewise, neoadjuvant hormone therapy has been shown to cause prominent T-cell infiltration of prostate tumors. Based on such findings, we asked whether radiation therapy (RT) and hormone therapy (HT), by causing tumor cell death in an inflammatory context, might induce tumor-specific immune responses in prostate cancer. Our preliminary results in the androgen-dependent murine Shionogi tumor model indicate that castration (the laboratory equivalent of HT) induces tumor-specific autoantibody responses in approximately 50% of animals. Moreover, parallel studies of human prostate cancer patients undergoing standard treatments at our institution indicate that HT and RT both induce tumor-specific autoantibody responses in up to 30% of patients, depending on the stage of disease and specific treatment. Based on these observations, we hypothesized that treatment-induced autoantibody responses in prostate cancer are accompanied by CD4+ and CD8+ T cell responses that potentially delay or prevent tumor recurrence.

This study has three specific aims:

Aim 1. To determine in the Shionogi mouse tumor model whether castration and brachytherapy induce autoantibody and T-cell responses that prevent or delay tumor recurrence.

Aim 2. To determine in human prostate cancer patients whether treatment-induced autoantibody responses are accompanied by tumor-specific CD4+ and CD8+ T-cell responses.

Aim 3. To determine whether tumor-specific autoantibody profiles differ in prostate cancer patients with recurrent versus non-recurrent disease.

BODY:

Aim 1. To determine in the Shionogi mouse tumor model whether castration and brachytherapy induce autoantibody and T-cell responses that prevent or delay tumor recurrence.

As described in the 2008 Annual Report, we identified poly(A) binding protein nuclear 1 (PABPN1) as the treatment-induced ~40 kDa antigen in our Shionogi model and used it to determine that autoantibody and T cell responses were associated with poorer outcomes in castrated mice bearing Shionogi tumors. A manuscript entitled “Castration induces autoantibody and T cell responses that correlate with inferior outcomes in an androgen-dependent murine tumor model” describing these results has recently been submitted to the

International Journal of Cancer for publication. A copy of this manuscript can be found in the Appendix.

We have also completed a preliminary CD4 depletion experiment in the castration only setting. A total of 15 mice were used for this experiment. Seven mice served as controls and were injected with PBS x days prior to tumor injection. Eight mice were injected with 400 μg of anti-CD4 antibody (clone GK1.5) at -3, -1, +3 days relative to castration. An additional 200 μg of depleting antibody was injected weekly post castration. All mice were castrated when tumors reached 65-100 mm2. Mice were re-injected with PBS or anti-CD4 antibody every 1 or 2 weeks. Flow cytometry on PBMCs from a subset of mice was performed to determine the efficacy of anti-CD4 depletion (Figure 1). PBMCs were stained with anti-CD3 to identify the T cell population (first panel, Figure 1 A-D) and with anti-CD4 (second panel, Figure 1 A-D). Results clearly show that the mice that were injected with PBS had healthy CD4+ T cell populations (~63% of the CD3+ population), whereas the CD4 depleted mice had virtually no CD4 T cells remaining (<1% of CD3+ T cells). We then compared the tumor growth between the PBS and CD4 depleted mice and found that there was a significant difference in the rate of recurrence between the two groups of mice (Figure 2). We will be repeating these experiments in the near future and expanding them to include depletion of the CD8+ T cells as well as CD19+ B cells. This will help us determine whether the poor outcomes seen in the castration only experiments are caused by the autoantibody or T cell immune response or whether the presence of PABPN1-specific autoantibodies and T cells are merely a marker of early tumor recurrence and poor prognosis.

Several additional mouse experiments were completed in the past year. While the experiments mentioned above focused only on castration, these experiments focused on the combination of castration and radiation therapy (in the form of brachytherapy) in the Shionogi model. These experiments were designed primarily to study the abscopal effect, described for ionizing radiation, where reduced tumour growth is observed outside of the field of radiation. It is thought that an immune-mediated mechanism is responsible for the abscopal effect.

The first set of castration+RT experiments was initiated to determine whether castration and brachytherapy led to delayed tumor recurrence of an untreated distal tumor. It was established in previous experiments that the number of I-125 radioactive seeds required to give optimal control of the primary tumor was 6, therefore this is the number of seeds used in the following experiments. Five experimental groups were established as follows:

Group ID	**Description**	**# Mice**
No Tx	No treatment	7
RT	six I-125 seeds only	7
Cx	Castration only	13
Cx+RT	Castration → Max. Regression → six I-125 seeds	13
Cx+RT@1/2	Castration → Partial Regression → six I-125 seeds	13

Cohorts of mice were implanted with two Shionogi tumors (one tumor per flank). No treatment was given in the No Tx group. In the RT group, 6 seeds were implanted in one tumor only once the tumor reached 65-100 mm^2. In the remaining groups, mice were castrated when tumors reached 65-100 mm^2. If the mice went on to receive radiation, seeds were implanted in only one (the primary) tumor at the point the tumor maximally regressed (Group Cx+RT) or when the

tumor partially regressed (Group Cx+RT@1/2). All mice were monitored for primary and distal tumor recurrence.

As expected, both primary and distal tumors in the No Tx group grew unchecked, whereas growth of primary tumors in the RT group was controlled. Distal tumors in the RT group grew at a rate similar to that seen in the No Tx group, indicating that no abscopal response was produced by RT alone in this setting. In the remaining groups, treated with castration alone or castration and radiation, radiation of the primary tumor had a clear effect on the rate of primary tumor growth, as expected (Figure 3A). Using Kaplan-Meier analysis we then compared the tumor-free survival between those three experimental groups (Figure 1B). As expected, when comparing primary tumor recurrence, addition of radiation leads to significant delays in tumor recurrence (Cx+RT vs Cx, $p<0.001$; Cx+RT@1/2 vs Cx, $p=0.0009$). When comparing distal tumor recurrence, evidence of an abscopal effect was seen in those mice who received radiation when their tumors regressed maximally after castration versus those that received only castration (Cx+RT vs Cx, $p=0.0096$) (Figure 3B). Interestingly, this abscopal effect was not seen in mice radiated when tumors only partially regressed after castration (Cx+RT@1/2 vs Cx, $p=0.54$), underscoring the importance of maximal tumor regression after castration. This is reminiscent of what is seen in the clinic with high-risk patients treated with HT+RT, where the best results are achieved if RT is given only after PSA nadir has been reached with HT.

The second set of castration+RT experiments included the use of Flt3 ligand, a potent stimulator of dendritic cells (DCs) and thought to be an important mediator of anti-tumour immune responses such as those needed for an abscopal effect. The hypothesis with this set of experiments was that administration of Flt3 ligand would enhance the immune-stimulatory effects of combined neo-adjuvant androgen withdrawal and brachytherapy (HT+BT) in mice to prevent or delay re-growth of the not only the primary irradiated tumour, but also the distal, non-irradiated tumour through an abscopal effect. To test the hypothesis 4 treatment groups were established as follows:

Group ID	**Description**	**# Mice**
Cx	Castration only	13
Cx+RT	Castration → Max. Regression → six I-125 seeds	13
Cx+Flt3L	Castration → Max. Regression → Flt3 ligand *	7
Cx+RT+Flt3L	Castration → Max. Regression → six I-125 seeds → Flt3 ligand **	19

* Flt3 ligand was injected once a day for 10 consecutive days 1 day following maximal regression of tumours

** Flt3 ligand was injected day 1 following implantation of I-125 seeds, for 10 consecutive days

Cohorts of mice were implanted with two Shionogi tumors (one tumor per flank). When tumors reached 65-100 mm^2, mice were castrated to induce regression of both tumors. When tumors had fully regressed, additional treatment was given as described above. In mice given brachytherapy, six seeds were implanted in one flank only at the site of the regressed tumor (primary tumor). All mice were monitored for recurrence of both the primary and distal tumors.

To confirm *in vivo* DC expansion, we performed flow cytometry analysis on blood samples taken from the different groups at time points during and post administration of Flt3 ligand and stained for mAb against dendritic cells (FITC-anti-CD11b and PE-anti-CD11c). As expected, we observed an expansion of dendritic cells in mice treated with Flt3 ligand compared to mice treated with castration only (13% vs. 3% of $CD11c^+$ cells, 59% vs 29% $CD11b^+$ cells).

We then compared the tumor-free survival of both the primary and distal tumor between the different treatment groups (Figure 4). Figure 4A demonstrates a direct anti-tumour response against the primary tumour to brachytherapy, Flt3 ligand and the combined treatment compared to castration alone, however there was no additional survival advantage using the combination treatment compared to brachytherapy alone. Figure 4B also demonstrates a slight anti-tumour effect against the distal tumor using I-125 seeds (graph Cx vs Cx+RT), supporting an abscopal response with therapeutic radiation. However, the combination of I-125 radiation and Flt3 ligand failed to demonstrate an abscopal response or enhancement of abscopal response. This may be due to the timing of administration of Flt3 ligand with respect to I-125 implantation. Additional experiments will be performed which will include a change in timing of Flt3 ligand administration to what we believe would be the time at which maximal DC expansion would occur, followed shortly by exposure to therapeutic radiation using I-125 radioisotopes.

With regard to the Statement of Work for Aim 1, we have completed the majority of the proposed experiments. The work that is left to complete includes performing the immunological assays (Western blots, T cell assays) for both Shionogi experiments described above to determine whether immune responses (autoantibody and/or T cell responses) are associated with poorer outcomes as was seen in the castration only experiments or if the addition of radiation therapy in this setting leads to improved outcomes as is seen in the clinical setting. In addition we plan on repeating the Flt3 ligand experiments with optimized timing of Flt3 ligand administration and repeating the depletion experiments to determine the immunological mechanism responsible for the poor outcomes seen in the castration only experiments. These experiments should be completed in 2009.

Aim 2. To determine in human prostate cancer patients whether treatment-induced autoantibody responses are accompanied by tumor-specific CD4+ and CD8+ T-cell responses.

In order to investigate tumor-specific T cell responses in human prostate cancer, we collected large (200 ml) blood samples from the 16 prostate cancer patients who showed treatment-induced antibody responses against specific antigens, with emphasis on the four patients in which the treatment-induced antigen was cloned. We were able to collect the large volume of blood from 11 of these patients including the 4 patients from whom antigens were cloned. PBMCs were isolated from these patients and frozen for use in T cell assays. The remaining 5 patients were contacted and agreed to donate a large volume of blood in the future when needed.

To ensure that the ELISPOT methodology was working we decided to test five of our HLA-A2+ patients with HLA-A2-restricted peptides from three well-known prostate cancer antigens, including PSA, PSCA and NY-ESO-1. We included 2 positive controls, PHA, which should stimulate T cells non-specifically, and CEF peptide, a pool of peptides from 3 different common viruses that most people have had exposure to. The results showed that of the 5 patients tested, none had T cells specific to the PSA, PSCA or NY-ESO-1 peptides. This is not unexpected as the frequency of T cells against these antigens is low amongst prostate cancer patients in general (Figure 5). Three patients were positive for T cells against the CEF peptide and both patients stimulated with PHA were also positive, indicating that the assay is working in our hands.

In order to avoid having to map the CD8+ epitopes for each of our antigens, we have established an *in vitro* transcribed mRNA platform (ivt RNA), in which the antigen of interest is expressed in autologous antigen presenting cells (APCs) which process and display peptides regardless of HLA haplotype. We are currently cloning 6 treatment-induced antigens as summarized below (Note that ZNF707/PTMA was a double insert clone, thus each needs to be cloned individually in order to ascertain which one is the treatment-induced antigen):

Antigen	Primers	Product Length (bp)	Status
PARP1	F: CAC CAT GGC GGA GTC TTC GGA TAA R: CCA CAG GGA GGT CTT AAA ATT GA	3042	In progress
SDCCAG1	F: CAC CAT GAA GAG CCG CTT TAG CAC R: TTT CCT TTT TAC GTT CAG AAG ATT GG	3228	In progress
ODF2	F: CAC CAT GTC TGC CTC ATC CTC AGG R: TCT TGG TAT GCG GGC C	1915	In progress
ZNF707	F: CAC CAT GGA CAT GGC CCA GGA R: CAC CTC CCC GTG CCT	1114	In progress
PTMA	F: CAC CAT GTC AGA CGC AGC CGT A R: GTC ATC CTC GTC GGT CTT CT	331	Cloned successfully
CEP78	F: CAC CAT GAT CGA CTC CGT GAA GCT R: GGA ATG CAG GTC CTT TCC AG	2166	In progress
SWAP70	F: CAC CAT GGG GAG CTT GAA GGA G R: CTC CGT GGT CTT TTT CTC TTT CC	1756	Cloned successfully

Once all the antigens are successfully cloned, they will be transfected into autologous B cells, which will act as APCs to allow *in vitro* transcription and presentation of peptides. ELISPOT assays will then be performed to determine whether antigen-specific T cells are present in these patients.

With regard to the Statement of Work for Aim 2, we will be concentrating our efforts on completing the T cell assays in the human patients. This should be completed by the summer of 2009. We are also planning to construct the yeast display library over the summer of 2009 to facilitate cloning of the remaining treatment-induced antigens identified by Western blot. We have deferred screening of the remaining 79 patients until analysis of the initial cohort is complete.

Aim 3. To determine whether tumor-specific autoantibody profiles differ in prostate cancer patients with recurrent versus non-recurrent disease.

We have continued to collect blood from prostate cancer patients treated ~5 years ago and who have since recurred (n=17) or not recurred (n=43). Recruitment of recurrent patients continues to be a challenge thus we have expanded our search to include patients diagnosed in 2003-2004, as this now represents the 5 year time frame. In addition, the subjects in our initial cohort of 174 prostate cancer patients are approaching the 5 year anniversary since treatment. Thus, these patients may provide an additional resource for this Aim, with the added benefit of being accompanied with pre-treatment blood draws. With regard to the Statement of Work, we expect that we will be able to complete recruitment of these two groups within 6 months. During this time, as mentioned above, we plan to construct the yeast display library and then begin analysis of serum samples at the end of 2009.

KEY RESEARCH ACCOMPLISHMENTS:

- Treatment-induced autoantibody responses are seen in approximately ~30% of prostate cancer patients undergoing hormone therapy and/or radiation therapy (Nesslinger et al. Clin Cancer Res 2007;13:1493-1502).

- Castration-induced autoantibody and T cell responses are associated with poor outcomes in the murine Shionogi tumor model (Hahn et al. Submitted to Int J Cancer, March 2009).

- The combination of castration and brachytherapy led to a delay in tumor recurrence, evidence of an abscopl response, in the murine Shionogi tumor model.

- An *in vitro* transcribed mRNA (ivt RNA) platform has been established to allow analysis of CD8+ T cell responses regardless of HLA haplotype.

REPORTABLE OUTCOMES:

Manuscripts:
Hahn S, Nesslinger NJ, Drapala RJ, Bowden M, Rennie PS, Pai HH, Ludgate C, Nelson BH. Castration induces autoantibody and T cell responses that correlate with inferior outcomes in an androgen-dependent murine tumor model. Submitted to Int J Cancer, March 2009.

Book chapters:
Nesslinger NJ, Pai HH, Ludgate CM, Nelson BH. Exploring the effects of standard treatments on the immune response to prostate cancer. 2009. *Methods of Cancer Diagnosis,Ttherapy, and Prognosis*, *Vol. 2.* Hayat, M.A. (Ed.).

Presentations:
Mapping and Manipulating the Immune Response to Cancer. Brad H. Nelson. City of Hope Medical Center, Duarte CA, March 2008.

Mapping and Manipulating the Immune Response to Cancer. Brad H. Nelson. University of Calgary, Calgary AB, April 2008.

Mapping and Manipulating the Immune Response to Cancer. Brad H. Nelson. MD Anderson Cancer Center, Houston TX, May 2008.

Toward personalized immunotherapy of cancer. Brad H. Nelson. Canadian Cancer Immunotherapy Workshop 2008, Toronto ON, Nov 2008.

Degrees Obtained:
Sara Hahn
Master of Science, Department of Biochemistry and Microbiology, University of Victoria
Thesis title: “The influence of host immunity on outcomes following hormone therapy for cancer”
Degree awarded: June 2008

CONCLUSION:

Both the murine and human portions of this study are progressing well and on schedule. The Shionogi experiments have produced some interesting and exciting results to date. We have recently submitted a manuscript detailing the castration only experiments in which we found that mice with autoantibody and T cell responses had poorer outcomes than those mice without an immune response. We have also found that the combination of castration and brachytherapy led to delayed tumor recurrence of a distal, unirradiated tumor in the Shionogi model, but only when the tumors had maximally regressed after castration. We are currently performing the immunological analyses to determine whether immune responses are associated with poor outcomes as was seen in the castration only experiments or whether the addition of brachytherapy leads to improved outcomes.

REFERENCES:

None

APPENDIX:

Manuscript: “Castration induces autoantibody and T cell responses that correlate with inferior outcomes in an androgen-dependent murine tumor model”

Please see following page for manuscript.

International Journal of Cancer

Castration induces autoantibody and T cell responses that correlate with inferior outcomes in an androgen-dependent murine tumor model

Journal:	*International Journal of Cancer*
Manuscript ID:	draft
Wiley - Manuscript type:	Tumor Immunology
Date Submitted by the Author:	
Complete List of Authors:	Hahn, Sara; BC Cancer Agency, Deeley Research Centre Nesslinger, Nancy; BC Cancer Agency, Deeley Research Centre Drapala, Robert; The Prostate Centre at Vancouver General Hospital Bowden, Mary; The Prostate Centre at Vancouver General Hospital Rennie, Paul; The Prostate Centre at Vancouver General Hospital Pai, Howard; BC Cancer Agency, Radiation Oncology-Vancouver Island Centre Ludgate, Charles; BC Cancer Agency, Radiation Oncology-Vancouver Island Centre Nelson, Brad; BC Cancer Agency, Deeley Research Centre
Key Words:	prostate cancer, immune response, tumor antigens, prognosis

Castration induces autoantibody and T cell responses that correlate with inferior outcomes in an androgen-dependent murine tumor model

SARA HAHN[1,2], NANCY J. NESSLINGER[3], ROBERT J. DRAPER[4], MARY BOWDEN[4], PAUL S. RENNIE[4], HOWARD H. PAI[3], CHARLES LUDGATE[3], BRAD H. NELSON[1,2]

[1]DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY, UNIVERSITY OF VICTORIA, VICTORIA

[2]TREV AND JOYCE DEELEY RESEARCH CENTRE, BRITISH COLUMBIA CANCER AGENCY, 2410 LE VICTORIA, BC, CANADA

[3]RADIATION ONCOLOGY PROGRAM, BC CANCER AGENCY-VANCOUVER ISLAND CENTRE, VICT CANADA

[4]PROSTATE CENTRE AT VANCOUVER GENERAL HOSPITAL, VANCOUVER, BC, CANADA

Grant support: PROSTATE CANCER RESEARCH FOUNDATION OF CANADA, U.S. DEPARTMENT O (W81XWH-07-1-0259), NSERC (CGS M – 317056 - 2005), BRITISH COLUMBIA CANCER FOUNDATION, AND THE CAPITAL REGION PROSTATE CENTRE (VICTORIA, BC).

Correspondence: DR. BRAD NELSON, BC CANCER AGENCY, 2410 LEE AVENUE, VICTORIA, BC, V8 6V5, CANADA. EMAIL: BNELSON@BCCANCER.BC.CA

ABSTRACT

WE RECENTLY REPORTED THAT HORMONE THERAPY INDUCES ANTIGEN-SPECIFIC AUTO RESPONSES IN PROSTATE CANCER PATIENTS. HOWEVER, THE CONTRIBUTION OF AUTOANTIBO CLINICAL OUTCOMES IS UNKNOWN. WE USED AN ANIMAL MODEL TO TEST THE HYPOTHESIS THERAPY-INDUCED IMMUNE RESPONSES MAY BE ASSOCIATED WITH DELAYED TUMOR RECU DD/S MICE BEARING ESTABLISHED TUMORS FROM THE ANDROGEN-DEPENDENT SHIONOGI CA WERE CASTRATED TO INDUCE TUMOR REGRESSION. TUMOR-SPECIFIC AUTOANTIBODY RESPO MEASURED BY IMMUNOBLOT, AND THE UNDERLYING ANTIGEN WAS IDENTIFIED BY SEROLO A CDNA EXPRESSION LIBRARY. T CELL RESPONSES WERE ASSESSED BY IMMUNOHISTOCHEMI ELISPOT. FOLLOWING CASTRATION, 97% OF MICE UNDERWENT COMPLETE TUMOR REGRESSI 72% EXPERIENCED TUMOR RECURRENCE 18-79 DAYS POST-CASTRATION, WHILE THE REMAINI REMAINED TUMOR-FREE FOR THE DURATION OF THE EXPERIMENT. IN 55% OF MICE, CASTRAT AUTOANTIBODY RESPONSES TO AN ANTIGEN IDENTIFIED AS POLY(A) BINDING PROTEIN NUCL CASTRATION ALSO INDUCED PABPN1-SPECIFIC T CELL RESPONSES, WHICH WERE HIGHLY COR AUTOANTIBODY RESPONSES, AND THIS WAS ACCOMPANIED BY DENSE INFILTRATION OF TUM CELLS 1-2 WEEKS AFTER CASTRATION. UNEXPECTEDLY, MICE THAT DEVELOPED AUTOANTIBO RESPONSES TO PABPN1 SHOWED A HIGHER RATE AND SHORTER LATENCY OF TUMOR RECURR WITH RECURRENT TUMORS, T CELL RESPONSES TO PABPN1 WERE STILL DETECTABLE; HOWEV INFILTRATES WERE RESTRICTED TO THE PERIPHERAL STROMA OF TUMORS. IN CONCLUSION, IMMUNE RESPONSES ARE ASSOCIATED WITH INFERIOR OUTCOMES IN THE SHIONOGI CARCIN RAISING CONCERNS ABOUT THE INFLUENCE OF TREATMENT-INDUCED IMMUNE RESPONSES O OUTCOMES IN HUMANS.

INTRODUCTION

PROSTATE CANCER IS THE MOST FREQUENTLY DIAGNOSED CANCER IN NORTH AMERIC
DESPITE IMPROVEMENTS IN EARLY DETECTION DUE TO PROSTATE-SPECIFIC ANTIGEN (PSA) S
REMAINS THE SECOND LEADING CAUSE OF CANCER-RELATED DEATH AMONG MEN[1,2]. STANDARD SURGICAL OR
RADIATION THERAPIES (RT) ARE USUALLY SUCCESSFUL IN CONTROLLING ORGAN CONFINED
TUMORS RECUR, THE DISEASE IS TYPICALLY SYSTEMIC AND HORMONE THERAPY (HT) REMAI
TREATMENT OPTION. WHILE HT IS INITIALLY EFFICACIOUS, PATIENTS EVENTUALLY PROGRE
RESISTANT DISEASE, WHICH IS INCURABLE[3]. THUS, THERE IS CLEAR NEED FOR IMPROVED TREATMENTS TO
PREVENT THE DEVELOPMENT OR OUTGROWTH OF ANDROGEN-INDEPENDENT TUMORS.

WHILE BOTH HT AND RT MEDIATE DIRECT KILLING OF TUMOR CELLS, THERE IS GROWI
THAT THESE TREATMENTS CAN ALSO INDUCE TUMOR-SPECIFIC IMMUNE RESPONSES[4]. RODEN
DEMONSTRATED THAT ANDROGEN DEPRIVATION IN TUMOR-FREE MALE MICE INCREASED TH
OF T CELLS RESIDING IN PERIPHERAL LYMPHOID TISSUES, AND ALSO LED TO TRANSIENT INCR
PROLIFERATION IN RESPONSE TO T CELL RECEPTOR STIMULATION. MOREOVER, HT INDUCES A
CELLS, AND THE RESULTING APOPTOTIC BODIES CAN SERVE AS AN EFFICIENT SOURCE OF AN
ANTIGEN PRESENTING CELLS (APCS). IN HUMANS[5], MERCADER *et al.* DEMONSTRATED THAT HT CAUSED
INCREASED LEVELS OF APCS EXPRESSING THE T CELL CO-STIMULATORY MOLECULES B7.1 AN
PROSTATE CANCER PATIENTS, WHICH WAS ACCOMPANIED BY PROFUSE INFILTRATION OF TU
T CELLS[6]. SIMILARLY, EXTERNAL BEAM RT INDUCES TUMOR CELL NECROSIS AND APOPTOSIS, W
ACCOMPANIED WITH INFLAMMATORY OR OTHER "DANGER" SIGNALS[7] THAT PROVIDE BOTH ANTIGE
AND MATURATION SIGNALS TO DENDRITIC CELLS[8-12]. THIS IN TURN LEADS TO THE INDUCTION
OF CD4+ AND CD8+ T CELL RESPONSES, WHICH CAN ULTIMATELY ELICIT AN ANTI-TUMOR EFF
WE HAVE RECENTLY SHOWN THAT HT AND RT INDUCE AUTOANTIBODY RESPONSES TO A VA
ASSOCIATED ANTIGENS IN 25-30% OF PROSTATE CANCER PATIENTS[12]. DESPITE THIS MOUNTING EVIDENCE THAT
HT AND RT INDUCE T AND B CELL RESPONSES, IT IS NOT YET KNOWN WHETHER THESE IMMU
INFLUENCE CLINICAL OUTCOMES.

THE MURINE SHIONOGI CARCINOMA MODEL (SC-115) IS A TRANSPLANTABLE ANDROGE
TUMOR THAT, DESPITE BEING OF MAMMARY ORIGIN, IS USED TO STUDY THE CONVERSION FR
DEPENDENT TO ANDROGEN-INDEPENDENT NEOPLASIA[14]. IN MALE BALB/C MICE, SHIONOGI TUMORS ARE ANDROGEN-
DEPENDENT SUCH THAT SURGICAL CASTRATION PRECIPITATES APOPTOSIS AND TUMOR REGR
REPRODUCIBLE MANNER, SIMILAR TO THAT SEEN AFTER ANDROGEN WITHDRAWAL IN HUMA

PATIENTS. HOWEVER, SIMILAR TO HUMAN PROSTATE CANCER, THE ANDROGEN-DEPLETED EI
RISE TO ANDROGEN-INDEPENDENT RECURRENT TUMORS IN ~30% OF MICE.[15-17] IN CONTRAST, SHIONOGI
TUMOR CELLS THAT SURVIVE HORMONE WITHDRAWAL, LIKE HUMAN PROSTATE TUMOR CEL
PROTEINS IMPLICATED IN CELL SURVIVAL AND PROGRESSION TO ANDROGEN INDEPENDENCE.[18] WE RECENTLY
SHOWED THAT CASTRATION INDUCES AUTOANTIBODY RESPONSES TO A ~40 KDA ANTIGEN IN
50% OF SHIONOGI TUMOR-BEARING MICE, WHICH IS REMINISCENT OF OUR FINDINGS IN HUMA
DISCUSSED ABOVE.[12] THUS, THE SHIONOGI MODEL PROVIDES AN EXPERIMENTAL SYSTEM FOR STU
RELATIONSHIP BETWEEN TREATMENT-INDUCED IMMUNE RESPONSES AND OUTCOMES. HERE,
MODEL TO TEST THE HYPOTHESIS THAT CASTRATION-INDUCED IMMUNE RESPONSES MAY BE
DELAYED TUMOR RECURRENCE.

MATERIALS AND METHODS

SEREX screening

SEREX SCREENING OF A PROSTATE CANCER PHAGE CDNA LIBRARY WAS CARRIED OUT
DESCRIBED[13] USING MOUSE SERA DILUTED 1/400 IN TBS/1% BSA. A DONKEY ANTI-MOUSE IGG AL
PHOSPHATASE-CONJUGATED ANTIBODY WAS USED FOR SECONDARY SCREENING (JACKSON I
LABORATORIES, WEST GROVE, PA).

Cloning and purification of SEREX-identified antigens

TO ISOLATE FULL-LENGTH CDNA CLONES FOR ANTIGENS IDENTIFIED BY SEREX, TOTAL
EXTRACTED FROM 1×10^7 SHIONOGI TUMOR CELLS USING THE RNEASY MINI KIT (QIAGEN, MISSISSAU
CANADA) AND THEN 0.08 µG TOTAL RNA WAS SYNTHESIZED INTO CDNA USING SUPERSCRIPT™ III
TRANSCRIPTASE (INVITROGEN, BURLINGTON, CANADA). PCR PRODUCTS WERE PURIFIED USIN
GEL EXTRACTION KIT (INVITROGEN, MISSISSAUGA, CANADA), CLONED INTO PENTR™/D-TOPO®
(INVITROGEN BURLINGTON, CANADA) AND TRANSFORMED INTO ONE SHOT® TOP10 CHEMICA
E. coli (INVITROGEN, BURLINGTON, CANADA). CLONES WERE VERIFIED BY SEQUENCING BEFO
INTO THE *E. coli* EXPRESSION VECTOR PDEST™17 (INVITROGEN, BURLINGTON, CANADA). CLONES WERE
TRANSFORMED INTO BL21-AI™ CELLS (INVITROGEN, BURLINGTON, CANADA), AND PROTEIN PRODUC
INDUCED BY ADDITION OF ARABINOSE. AFTER 2 HOURS, BACTERIAL PELLETS WERE RE-SUSPE
MM TRIS-HCL, PH 7.5, 500 MM NACL, 20 MM IMIDAZOLE, AND 1 MM DITHIOTHREITOL. AFTER O
FREEZE-THAW CYCLE AT -80 ºC, BACTERIA WERE SONICATED AND CENTRIFUGED. SUPERNAT

WERE ANALYZED BY SDS-PAGE, AND FRACTIONS CONTAINING THE MOST PROTEIN WERE LOA
HITRAP IMAC FF NICKEL COLUMN (GE HEALTHCARE, PISCATAWAY, NJ) AND PURIFIED BY IMM
METAL ION ADSORPTION CHROMATOGRAPHY (IMAC) USING THE ÄKTA™ FPLC (GE HEALTHCARE,
PISCATAWAY, NJ). PROTEINS WERE ELUTED BY IMIDAZOLE GRADIENT. FRACTIONS WERE PO
DIALYZED AGAINST 2 L OF PHOSPHATE BUFFERED SALINE (PBS) OVERNIGHT AT 4ºC.

Shionogi mouse model

MICE WERE MAINTAINED AT THE ANIMAL CARE UNIT OF THE JACK BELL CENTRE. ALL
FOLLOWED THE GUIDELINES OF THE CANADIAN COUNCIL FOR ANIMAL CARE AND WERE APPI
ANIMAL CARE ADVISORY COMMITTEE OF THE UNIVERSITY OF BRITISH COLUMBIA. ADULT MA
WERE INJECTED SUBCUTANEOUSLY IN THE NECK REGION WITH 5 X 10 SHIONOGI CARCINOMA CELLS. WHEN
TUMORS REACHED ~8 MM X 10 MM IN SIZE, MICE WERE CASTRATED TO INDUCE ANDROGEN D
SUBSEQUENT TUMOR REGRESSION. SERIAL BLOOD SAMPLES WERE COLLECTED FROM THE TA
INOCULATION, BEFORE CASTRATION, AND THEN TWICE WEEKLY FOLLOWING CASTRATION. T
WIDTH) WAS MEASURED USING MICRO CALIPERS. TUMORS WERE CONSIDERED TO HAVE RECU
PALPABLE. UNLESS OTHERWISE INDICATED, MICE WERE SACRIFICED WHEN RECURRENT TUM
APPROXIMATELY 10% OF TOTAL BODY WEIGHT. ON NECROPSY, TERMINAL BLOOD SAMPLES
BY CARDIAC PUNCTURE. TUMORS WERE REMOVED AND DIVIDED IN TWO HALVES, WHICH WE
IN LIQUID NITROGEN OR FIXED IN 10% FORMALIN. LYMPH NODES AND SPLEEN WERE PROCES
SINGLE-CELL SUSPENSIONS USING THE BLUNT END OF A 5 MM SYRINGE AND A 40-µM CELL ST
SPLENOCYTES WERE RE-SUSPENDED IN ACK LYSIS BUFFER (0.15 M NH4Cl, 1 MM KHCO3, 0.1 MM
EDTA, PH 7.3). LYMPHOCYTES AND SPLENOCYTES WERE COMBINED, COUNTED, AND FROZEN
50%FBS/10%DMSO FOR LONG-TERM STORAGE.

Immunoblotting of tumor lysates

CYTOPLASMIC PROTEIN LYSATE WAS MADE FROM INTACT SHIONOGI TUMORS POOLED
CASTRATED MICE. FROZEN TUMORS WERE PULVERIZED INTO A FINE POWDER IN LIQUID NITR
SUSPENDED IN LYSIS BUFFER (1X DULBECCO'S PBS, 0.01% TRITON, PROTEASE INHIBITOR COCK
HOMOGENIZED THROUGH 18G AND 21G NEEDLES, AND THEN SONICATED. ALIQUOTS CONTAI
PROTEIN LYSATE WERE IMMUNOBLOTTED WITH MOUSE SERUM (1/500) FOLLOWED BY HRP-CO
ANTI-MOUSE IGG (H+L; JACKSON IMMUNORESEARCH, WEST GROVE, PA) AS DESCRIBED PREVI

Flow cytometry

TO ISOLATE TUMOR-INFILTRATING LYMPHOCYTES, TUMOR FRAGMENTS WERE PRESSE
µM MEMBRANE WITH THE BLUNT END OF A 5 MM SYRINGE, AND THE RESULTING CELL SUSPE
CENTRIFUGED AND RE-SUSPENDED IN 0.5 ML OF 1.0% BSA/PBS. CELLS WERE STAINED IN VARI
COMBINATIONS WITH THE FOLLOWING FLUOROCHROME-CONJUGATED ANTIBODIES AT 1/400
BSA/PBS: CD3-FITC, CD4-PE, CD4-PERCP, CD8-CY-CHROME, AND CD44-PE (BECTON,
DICKINSON AND COMPANY, OAKVILLE, ON). ISOTYPE-MATCHED FLUOROCHROME-CONJUGAT
SERVED AS NEGATIVE CONTROLS. CELLS WERE ANALYZED ON A BD FACSCALIBUR™
SYSTEM (BECTON, DICKINSON AND COMPANY, OAKVILLE, CANADA) WITH FLOWJO SOFTWAR
INC., ASHLAND, OR).

IFN-γ ELISPOT assay

96-WELL MULTISCREEN-HA, 0.45 µM FILTER PLATES (MILLIPORE, BILLERICA, MA) WERE PRE-WET
WITH 70% ETHANOL FOLLOWED BY THREE WASHES WITH STERILE PBS. PLATES WERE INCUBA
4ºC WITH 50 µL/WELL ANTI-MOUSE IFN-γ (10 µG/ML; MABTECH, MARIEMONT, OH). AFTER THREE
PBS WASHES, PLATES WERE BLOCKED WITH T CELL MEDIA (RPMI-1640 SUPPLEMENTED WITH
MM SODIUM PYRUVATE, 2 MM L-GLUTAMINE, 100 µG/ML PENICILLIN/STREPTOMYCIN, AND 25
MERCAPTOETHANOL) FOR 2 HOURS AT 37ºC. SPLENOCYTES WERE ADDED TO EACH WELL. PABPN1
PROTEIN WAS ADDED TO A FINAL CONCENTRATION OF 10 µG/ML. CONA WAS ADDED TO A FIN
OF 2 µG/ML. T CELL MEDIA WAS USED AS A NEGATIVE CONTROL. SAMPLES WERE RUN IN TRI
WERE INCUBATED FOR AT LEAST 20 HOURS AT 37 ºC. AFTER WASHING SIX TIMES WITH PBS/0.
20, 100 µL OF BIOTINYLATED ANTI-MOUSE IFN-γ (CLONE XMG1.2) DILUTED TO 1 µG/ML IN 0.5% BSA/PBS/0.05%
TWEEN-20; MABTECH, R4-6A2, MARIEMONT, OH) WAS ADDED TO EACH WELL. PLATES WERE L
HOURS AT 37 ºC AND THEN WASHED 12 TIMES WITH PBS/0.05% TWEEN-20. AVIDIN PEROXIDASE
COMPLEX (VECTOR LABORATORIES, BURLINGAME, CA) (100 µL/WELL) WAS ADDED FOLLOWE
INCUBATION AT ROOM TEMPERATURE. PLATES WERE WASHED AS ABOVE AND THEN DEVELO
VECTASTAIN AEC SUBSTRATE KIT (VECTOR LABORATORIES, BURLINGAME, CA) FOR APPROXI
MINUTES. DEVELOPMENT WAS STOPPED BY RINSING WITH TAP WATER. AIR-DRIED PLATES W
ZELLNET CONSULTING, INC. (FORT LEE, NJ) FOR ENUMERATION OF SPOTS USING AN AUTOMA
READER WITH KS ELISPOT SOFTWARE 4.9 (CARL ZEISS, THORNWOOD, NY).

Immunohistochemistry

FORMALIN-FIXED TUMORS WERE PROCESSED FOLLOWING STANDARD METHODS AND ST
HEMATOXYLIN AND EOSIN (H&E). A TISSUE MICROARRAY (TMA) CONTAINING ALL EXPERIME
WAS CONSTRUCTED USING DUPLICATE 1 MM CORES AND STAINED WITH MOUSE MONOCLONA
AGAINST CD3 (LAB VISION, RM9107, FREMONT, CA), FOXP3 (EBIOSCIENCE, 14-5773, SAN DIEGO,
CA), PAX-5 (LAB VISION, RB9406, FREMONT, CA), AND GRANZYME B (ABCAM, AB4059, CAMBRID
MA). SCORING OF THE TMA WAS PERFORMED INDEPENDENTLY BY TWO INDIVIDUALS WHO W
AS TO THE EXPERIMENTAL STATUS OF TUMORS. A SCORE OF 0 (NO INFILTRATION) TO 3 (DENS
ASSIGNED TO EACH TUMOR, AND SCORES WERE AVERAGED. A SCORE OF GREATER THAN 1 W
POSITIVE FOR LYMPHOCYTE INFILTRATION.

RESULTS

Castration induces autoantibody responses to PABPN1 in the Shionogi carcinoma model

WE PREVIOUSLY REPORTED THAT CASTRATION OF DD/S MICE BEARING SHIONOGI TUMO
IGG AUTOANTIBODY RESPONSES TO AN UNIDENTIFIED ~40 KDA ANTIGEN IN APPROXIMATELY
[13]. TO FACILITATE CLONING OF THIS ANTIGEN BY SEREX, WE FIRST DETERMINED WHETHER TH
ANTIGEN MIGHT HAVE A HUMAN HOMOLOG, AS THIS WOULD ALLOW USE OF A PREVIOUSLY C
EXPRESSION LIBRARY DERIVED FROM HUMAN PROSTATE CANCER CELL LINES [13]. IMMUNOBLOTTING
IMMUNOBLOTTED LYSATE FROM THE HUMAN PROSTATE CANCER CELL LINE LNCAP WITH SE
BEARING, CASTRATED MICE THAT WERE POSITIVE FOR AUTOANTIBODIES TO THE ~40 KDA AN
SEROREACTIVE BAND WAS SEEN AT ~40 KDA, INDICATING THE ANTIGEN SEEN IN SHIONOGI TU
INDEED HAVE A HUMAN HOMOLOG (DATA NOT SHOWN). TO IDENTIFY THE ANTIGEN, WE SCR
APPROXIMATELY 2.3×10^4 CLONES OF THE HUMAN PROSTATE CDNA EXPRESSION LIBRARY WITH MO
THAT WERE POSITIVE FOR AUTOANTIBODIES TO THE ~40 KDA ANTIGEN. FOUR SEROLOGICALL
ANTIGENS WERE CLONED. THE CORRESPONDING RECOMBINANT PROTEINS WERE IMMUNOBL
ADDITIONAL MOUSE SERA THAT WERE KNOWN TO BE POSITIVE OR NEGATIVE FOR AUTOANTI
KDA ANTIGEN. THE PATTERN OF SEROREACTIVITY TO ONE ANTIGEN, POLY(A) BINDING PROTE
(PABPN1), WAS IDENTICAL TO THE PATTERN OF SEROREACTIVITY TO THE ~40 KDA ANTIGEN F
TUMOR LYSATES (FIGURE 1A), SUGGESTING PABPN1 WAS THE CORRECT ANTIGEN.

TO CONFIRM THAT PABPN1 WAS THE ~40 KDA ANTIGEN IDENTIFIED IN SHIONOGI TUMO
HVE MALE MICE WERE IMMUNIZED WITH RECOMBINANT PABPN1, AND SERA FROM IMMUNIZE
WERE USED TO PROBE SHIONOGI TUMOR LYSATE. THE PRESENCE OF A STRONG IMMUNOREAC
KDA CONFIRMED THAT PABPN1 WAS INDEED THE 40 KDA ANTIGEN (FIGURE 1B).

BY WESTERN BLOT, PABPN1 WAS EXPRESSED AT HIGH LEVELS IN SHIONOGI TUMOR LY
AS NORMAL LIVER, LUNG AND UTERINE MOUSE TISSUES. BY CONTRAST, PABPN1 WAS NOT EX
NORMAL KIDNEY, SKELETAL MUSCLE OR HEART (FIGURE 1C). THIS IS IN ACCORD WITH PUBLI
HUMAN TISSUES, WHERE PABPN1 IS EXPRESSED AT HIGHER LEVELS IN LIVER, LUNG, AND UTE
TO KIDNEY, MUSCLE, AND HEART [19]

Autoantibody and T cell responses to PABPN1

TO ESTABLISH THE TIME COURSE OF AUTOANTIBODY RESPONSES TO PABPN1, A COHORT
BEARING ESTABLISHED SHIONOGI TUMORS WERE CASTRATED, AND SERIAL BLOOD SAMPLES
AUTOANTIBODY RESPONSES TO PABPN1 BY IMMUNOBLOTTING. OVERALL, 18/33 MICE (54.5%)
AUTOANTIBODY RESPONSE TO PABPN1, WHICH APPEARED AN AVERAGE OF 26 DAYS POST-CA
6-47 DAYS POST-CASTRATION). BY CONTRAST, AUTOANTIBODIES TO PABPN1 WERE NOT FOUN
FROM TUMOR-BEARING, NON-CASTRATED MICE OR CASTRATED NON-TUMOR-BEARING MICE

THE FACT THAT AUTOANTIBODIES TO PABPN1 WERE OF THE IGG SUBCLASS SUGGESTE
OF AN UNDERLYING T CELL RESPONSE. THIS WAS INVESTIGATED BY ELISPOT ANALYSIS OF
SPLENOCYTES. AS EXPECTED, WILD-TYPE MICE IMMUNIZED WITH RECOMBINANT PABPN1 SH
T CELL RESPONSES TO PABPN1, WHEREAS NON-IMMUNIZED CONTROL MICE SHOWED NO RES
1D). OF 19 TUMOR-BEARING CASTRATED MICE EXAMINED, 7 (36.8%) SHOWED A STRONG T CEL
TO PABPN1. ALL OF THESE MICE ALSO HAD AN AUTOANTIBODY RESPONSE TO PABPN1. CONVE
12 MICE THAT WERE NEGATIVE FOR T CELL RESPONSES TO PABPN1, 11 WERE ALSO NEGATIVE
AUTOANTIBODIES TO PABPN1. THUS, THERE WAS HIGH CONCORDANCE BETWEEN AUTOANTIB
RESPONSES IN TUMOR-BEARING, CASTRATED MICE. HOWEVER, CONTROL MICE SHOWED A LO
BETWEEN AUTOANTIBODY AND T CELL RESPONSES. SPECIFICALLY, NON-TUMOR-BEARING MI
CASTRATION SHOWED MODEST T CELL RESPONSES TO PABPN1 (FIGURE 1D). THUS, CASTRATIO
INDUCE T CELL RESPONSES TO PABPN1, BUT THE EFFECT IS ENHANCED IN TUMOR-BEARING M

PABPN1 antibody and T cell responses are associated with early tumor recurrence

WE NEXT EXAMINED THE RELATIONSHIP BETWEEN CASTRATION-INDUCED AUTOANTIB
AND TUMOR RECURRENCE. FOLLOWING CASTRATION, 32/33 MICE EXPERIENCED COMPLETE T
OF THESE 32 MICE, 72% (23/32) EXPERIENCED TUMOR RECURRENCE 18-79 DAYS POST-CASTRAT
THE REMAINING 28% (9/32) REMAINED TUMOR-FREE FOR THE DURATION OF THE EXPERIMENT
POST-CASTRATION) AFTER WHICH THEY WERE SACRIFICED FOR ANALYSIS. SEVENTY PERCEN
WITH RECURRENT TUMORS HAD AN AUTOANTIBODY RESPONSE TO PABPN1, COMPARED TO O
MICE THAT REMAINED TUMOR-FREE (P=0.005, FISHER'S EXACT TEST). ACCORDINGLY, THE ME
INTERVAL FOR MICE WITH AUTOANTIBODIES TO PABPN1 WAS 24.5 DAYS COMPARED TO 62.9 D
WITHOUT AUTOANTIBODIES TO PABPN1 (P<0.0001, TWO-TAILED UNPAIRED T-TEST).

T CELL RESPONSES TO PABPN1 WERE ASSESSED BY IFN-γ ELISPOT USING SPLENOCYTES
HARVESTED AT THE TIME OF EUTHANASIA. AS BEFORE, MICE WITH AUTOANTIBODIES TO PAB
STRONGER T CELL RESPONSES TO PABPN1 (MEAN = 406 SPOT-FORMING CELLS/10^6 SPLENOCYTES, N=8) THAN
MICE WITHOUT AUTOANTIBODIES TO PABPN1 (MEAN = 38 SPOT-FORMING CELLS/10^6 SPLENOCYTES, N=10).
ACCORDINGLY, MICE WITH RECURRENT TUMORS HAD STRONGER PABPN1-SPECIFIC T CELL RI
= 335 SPOT-FORMING CELLS/10^6 SPLENOCYTES, N=10) THAN MICE WITHOUT RECURRENT TUMORS (ME
SPOT-FORMING CELLS/10^6 SPLENOCYTES, N=9). KAPLAN-MEIER ANALYSIS REVEALED A SIGNIFICANT
DIFFERENCE IN TIME TO RECURRENCE BETWEEN MICE WITH AND WITHOUT PABPN1 ANTIBOD
RESPONSES (FIGURE 2). THUS, CONTRARY TO OUR INITIAL HYPOTHESIS, TREATMENT-INDUCE
AND T CELL RESPONSES TO PABPN1 WERE CORRELATED WITH INFERIOR OUTCOMES.

TO INVESTIGATE WHETHER AUTOANTIBODIES TO PABPN1 PROMOTE TUMOR RECURREN
SERVE AS A MARKER OF RECURRENCE, WE ASSESSED THE TIMING OF AUTOANTIBODY RESPO
TUMOR RECURRENCE. IN 8 OF 16 MICE WHOSE TUMORS RECURRED, THE AUTOANTIBODY RES
DETECTED 5 OR MORE DAYS PRIOR TO TUMOR RECURRENCE. HOWEVER, IN 6 OF 16 MICE, THE
RESPONSE WAS NOT DETECTED UNTIL 2 OR MORE DAYS AFTER TUMOR RECURRENCE, MAKIN
AUTOANTIBODIES CONTRIBUTED DIRECTLY TO RECURRENCE. IN THE REMAINING 2/16 MICE,
RESPONSE WAS DETECTED WITHIN ONE DAY OF TUMOR RECURRENCE, SUCH THAT THE TEMP
COULD NOT BE RELIABLY DISCERNED. OVERALL, THESE DATA ARE CONSISTENT WITH THE N
AUTOANTIBODIES ARE A MARKER OF TUMOR RECURRENCE RATHER THAN AN ESSENTIAL ME

THIS FINDING LED US TO CONSIDER WHAT OTHER FEATURES OF TUMORS MIGHT CORRE
AUTOANTIBODY STATUS. THE AVERAGE TUMOR SIZE AT CASTRATION FOR THOSE MICE THAT V
AN AUTOANTIBODY RESPONSE WAS 82.4 MM^2 AS COMPARED TO 79.7 MM^2 FOR THOSE WITH NO RESPONSE,
SUGGESTING THAT THE SIZE OF THE PRIMARY TUMOR DID NOT INFLUENCE SUBSEQUENT AUT
DEVELOPMENT (P=0.4963, TWO-TAILED UNPAIRED T-TEST). WE THEN CONSIDERED THE RATE
REGRESSION AFTER CASTRATION. IN THOSE MICE THAT DEVELOPED AN AUTOANTIBODY RES
AVERAGE OF 9.7 DAYS FOR TUMORS TO REGRESS AFTER CASTRATION COMPARED TO 6.8 DAYS
DID NOT DEVELOP AN AUTOANTIBODY RESPONSE (P=0.049, TWO-TAILED UNPAIRED T-TEST). A
TEMPORAL DIFFERENCE WAS ONLY 3 DAYS, THIS NONETHELESS SUGGESTS THAT SLOWLY RE
MAY BE MORE LIKELY TO TRIGGER AUTOANTIBODY RESPONSES AND, ULTIMATELY, TO RECU

THE UNEXPECTED FINDING THAT PABPN1-SPECIFIC AUTOANTIBODY AND T CELL RESPO
ASSOCIATED WITH INFERIOR OUTCOMES RAISED THE ISSUE OF WHETHER RECURRENT SHIONO
EXPRESSED THE TARGET ANTIGEN PABPN1. WE EVALUATED THIS ISSUE BY IMMUNOBLOTTIN
TUMORS FOR EXPRESSION OF PABPN1. ALL RECURRENT TUMORS EXPRESSED PABPN1, AND IN
THE LEVEL EXPRESSION WAS SIMILAR TO PRIMARY TUMOR (FIGURE 3).

T cell infiltration of Shionogi tumors after castration and upon recurrence.

SINCE MICE WITH RECURRENT TUMORS HAD SUBSTANTIAL PABPN1-SPECIFIC T CELL RE
TUMORS STILL EXPRESSED ANTIGEN, WE NEXT INVESTIGATED WHETHER T CELLS TRAFFICKE
THE TUMOR SITE. TO THIS END, A TIME COURSE EXPERIMENT WAS PERFORMED IN WHICH 50 T
MICE WERE CASTRATED AND THEN GROUPS OF 10 MICE WERE EUTHANIZED ON DAY 7 OR 14 (
WERE REGRESSING); DAY 28 OR 35 (WHILE TUMORS WERE RECURRING); OR AT LATER TIME PO
RECURRENT TUMORS REACHED ~10% OF THE BODY WEIGHT. AN ADDITIONAL 10 TUMOR-BEAR
NOT CASTRATED. BY IHC, TUMORS FROM NON-CASTRATED MICE HAD VERY FEW CD3+ T CELL
EPITHELIUM OR STROMA (FIGURE 4A- NON-CASTRATED). IN CONTRAST, ON DAYS 7 AND 14 PO
12/18 TUMORS WERE DENSELY INFILTRATED BY CD3+ T CELLS (FIGURE 4A- DAY 7 AND DAY 14
FACS ANALYSIS AVERAGED OVER THREE EXPERIMENTS, ~55% OF TUMOR-INFILTRATING CD3+
CD8+, AND ~24% WERE CD4+ T CELLS. OVER 95% OF T CELLS WERE CD44^{HIGH} EXPRESSING AN
ACTIVATED PHENOTYPE (DATA NOT SHOWN). BY CONTRAST, VERY FEW TUMOR-INFILTRATIN
GRANZYME B AS ASSESSED BY IHC, SUGGESTING A PAUCITY OF MATURE CYTOLYTIC EFFECTO[20].
TUMORS WERE ALSO EXAMINED BY IHC FOR CELLS EXPRESSING FOXP3, A TRANSCRIPTION FA

BY REGULATORY T CELLS (TREGS)[21]. VERY FEW FOXP3+ CELLS WERE SEEN IN TUMORS FROM NON-CAST
MICE. IN CASTRATED MICE, THE NUMBER OF TUMOR-INFILTRATING FOXP3+ CELLS WAS PROP
NUMBER OF CD3+ T CELLS (I.E., ABOUT 5-10%) (FIGURE 4B). FINALLY, TUMORS FROM CASTRAT
CASTRATED MICE CONTAINED VERY FEW B CELLS, AS ASSESSED BY IHC WITH AN ANTIBODY
NOT SHOWN)[22].

IN CONTRAST TO THE REGRESSING TUMORS DESCRIBED ABOVE, RECURRENT TUMORS
RELATIVELY SPARSE CD3+ T CELL INFILTRATES IN TUMOR EPITHELIUM (FIGURE 4A- DAY 28).
FEW CELLS EXPRESSING FOXP3, GRANZYME B OR PAX-5 WERE SEEN (FIGURE 4B- DAY 28 AND
SHOWN). HOWEVER, THE PERIPHERAL AND STROMAL REGIONS OF RECURRENT TUMORS SHOW
ACCUMULATIONS OF CD3+ T CELLS (FIGURE 4C). THUS, IT APPEARS THAT RECURRENT SHIONO
AVOID IMMUNE REJECTION BY PREVENTING THE INFILTRATION OF T CELLS INTO TUMOR EPIT

DISCUSSION

WE RECENTLY SHOWED THAT HORMONE AND RADIATION THERAPY INDUCE ANTIGEN-
AUTOANTIBODY RESPONSES IN A SIGNIFICANT PROPORTION OF HUMAN PROSTATE CANCER P[13]. HOWEVER,
IT IS NOT YET KNOWN HOW THESE TREATMENT-ASSOCIATED IMMUNE RESPONSES CORRELAT
OUTCOMES. TO ADDRESS THIS QUESTION EXPERIMENTALLY, WE UTILIZED THE MURINE SHIO
MODEL, WHICH EXHIBITS TREATMENT-INDUCED AUTOANTIBODY RESPONSES SIMILAR TO TH
PROSTATE CANCER PATIENTS. WE HYPOTHESIZED THAT TREATMENT-INDUCED AUTOANTIBO
BE ASSOCIATED WITH DELAYED TIME TO TUMOR RECURRENCE AND PROLONGED SURVIVAL.
HYPOTHESIS, WE FIRST CLONED PABPN1, THE ANTIGEN THAT UNDERLIES TREATMENT-INDUC
RESPONSES IN THIS MODEL. IN GENERAL, MICE THAT DEVELOPED PABPN1-SPECIFIC AUTOANT
CASTRATION ALSO DEVELOPED PABPN1-SPECIFIC T CELL RESPONSES. CONTRARY TO OUR HY
DEVELOPMENT OF PABPN1-SPECIFIC AUTOANTIBODY AND T CELL RESPONSES WAS ASSOCIAT
RAPID AND FREQUENT TUMOR RECURRENCES. MICE WITH RECURRENT TUMORS RETAINED R
AND T CELL RESPONSES TO PABPN1, AND PABPN1 WAS STILL EXPRESSED AT HIGH LEVELS BY
NOTABLY, HOWEVER, RECURRENT TUMORS HAD GREATLY REDUCED LYMPHOCYTIC INFILTR
CELLS BEING RESTRICTED TO PERIPHERAL STROMAL REGIONS. THESE FINDINGS RAISE CONC
INDUCED IMMUNE RESPONSES MAY HAVE A NEGATIVE IMPACT ON CLINICAL OUTCOMES IN P
PATIENTS.

BASED ON OUR RESULTS, WE CONSIDERED THE POSSIBILITY THAT TREATMENT-INDUCE
AND T CELL RESPONSES MIGHT SOMEHOW PROMOTE TUMOR RECURRENCE. FOR EXAMPLE, B C
SHOWN TO PLAY AN ESSENTIAL ROLE IN INFLAMMATION-INDUCED TUMORIGENESIS IN A MU
CANCER MODEL[23]. FURTHERMORE, T CELLS CAN PROMOTE CARCINOGENESIS IN THE SETTING OF C
INFLAMMATION. FOR EXAMPLE, IN CHRONIC HEPATITIS B AND C INFECTION, CYTOTOXIC LYM
PROMOTE HEPATOCYTE DAMAGE AND FIBROSIS THROUGH DIRECT CELLULAR TOXICITY AND
INFLAMMATORY CYTOKINES[24]. LIKEWISE, T CELLS DIRECTED AGAINST HELICOBACTER PYLORI ARE T
PROMOTE THE DEVELOPMENT OF GASTRIC ADENOCARCINOMA[25]. HOWEVER, THE SHIONOGI MODEL DOES NOT
INVOLVE CHRONIC INFLAMMATION NOR PRIMARY CARCINOGENESIS, THEREFORE THE RELEV
EXAMPLES IS UNCLEAR. IN 6/16 CASES FROM OUR STUDY, AUTOANTIBODY RESPONSES TO PA
AFTER RECURRENT TUMORS WERE DETECTED, WHICH SEEMS INCOMPATIBLE WITH THE NOTI
AUTOANTIBODIES PLAY A CAUSATIVE ROLE IN TUMOR RECURRENCE. AN ALTERNATIVE POSS
AUTOANTIBODIES ARE A MARKER RATHER THAN MEDIATOR OF TUMOR RECURRENCE. INDEE
OF AUTOANTIBODIES TO PABPN1 WAS ASSOCIATED WITH TUMORS THAT REGRESSED MORE S
CASTRATION. IT MAY BE THAT A SLOWER RATE OF TUMOR REGRESSION ALLOWS SUFFICIENT
PRESENTATION TO THE IMMUNE SYSTEM, RESULTING IN AUTOANTIBODY AND T CELL RESPO
REGRESSING TUMORS MAY ALSO BE LESS ANDROGEN-DEPENDENT AND HENCE MORE LIKELY
MODEL PROVIDES A PLAUSIBLE, INDIRECT MECHANISM LINKING AUTOANTIBODIES TO TUMO
FUTURE STUDIES WILL DIRECTLY TEST THESE POSSIBILITIES BY DEPLETING B AND T CELL SU
AND ASSESSING THE AFFECT ON TUMOR RECURRENCE.

IN ADDITION TO AUTOANTIBODIES, WE ALSO DETECTED ROBUST T CELL RESPONSES T
IFN-γ ELISPOT. INTRIGUINGLY, MODEST T CELL RESPONSES TO PABPN1 WERE ALSO SEEN IN TU
MICE THAT UNDERWENT CASTRATION. CASTRATION HAS BEEN SHOWN TO INCREASE LEVELS
PERIPHERAL LYMPHOID TISSUES OF MICE DUE TO A LOSS OF THE IMMUNE SUPPRESSIVE EFFE
[4]. THUS, CASTRATION-INDUCED T CELL RESPONSES TO PABPN1 MAY IN PART REFLECT THE GE
HOMEOSTATIC EXPANSION OF LYMPHOCYTES IN RESPONSE TO ANDROGEN DEPRIVATION. TH
STRONGER T CELL RESPONSES TO PABPN1 DEVELOP IN TUMOR-BEARING MICE SUGGESTS THA
APOPTOSIS FURTHER STIMULATES PABPN1-SPECIFIC T CELLS THROUGH THE RELEASE OF SOL
AND/OR INFLAMMATORY FACTORS. CASTRATION-INDUCED T CELL RESPONSES WERE ACCOM
AND DENSE INFILTRATION OF TUMOR EPITHELIUM BY CD3+ T CELLS BY DAY 7. THE MAJORIT
THESE T CELLS EXPRESSED CD8, A SMALLER PROPORTION (24%) EXPRESSED CD4+, AND A MIN

POPULATION (5-10%) EXPRESSED FOXP3. INTRIGUINGLY, HORMONE THERAPY OF HUMAN PRO
ALSO PROMOTES T CELL INFILTRATION OF TUMORS, ALTHOUGH THE CLINICAL SIGNIFICANCE
IS NOT KNOWN[6]. LIKEWISE, IN THE SHIONOGI MODEL, WE WERE UNABLE TO ASSESS THE RELATIO
BETWEEN T CELL INFILTRATION OF TUMORS AND SUBSEQUENT OUTCOMES, SINCE MICE HAD
ON DAYS 7-14 IN ORDER FOR T CELL INFILTRATION TO BE ASSESSED. HOWEVER, WHEN RECUR
WERE ANALYZED, T CELLS NO LONGER INFILTRATED TUMOR EPITHELIUM, BUT RATHER WER
PERIPHERAL STROMAL REGIONS. COLLECTIVELY, OUR RESULTS INDICATE THAT SHIONOGI TU
PERMISSIVE TO T CELL INFILTRATION, WHEREAS RECURRENT TUMORS DEVELOP LYMPHOCYT
BARRIERS. THIS SUGGESTS THAT THE T CELL RESPONSE TRIGGERED BY CASTRATION MAY CR
PRESSURE FOR THE DEVELOPMENT OF IMMUNOLOGICALLY RESISTANT TUMORS UPON RECUR
INTERESTING TO ASSESS WHETHER RECURRENT HUMAN PROSTATE TUMORS ARE SIMILARLY

PABPN1 IS A UBIQUITOUSLY EXPRESSED PROTEIN THAT IS INVOLVED IN THE POLYADEN
MRNA IN EUKARYOTES. ALTHOUGH PABPN1 HAS NOT BEEN DIRECTLY IMPLICATED IN CANCE
EXPANSION OF THE TRINUCLEOTIDE REPEAT IN A POLYALANINE TRACT OF THE PABPN1 GENE
OCULOPHARYNGEAL MUSCULAR DYSTROPHY, AN AUTOSOMAL DOMINANT INHERITED DISOR[26].
THE ROLE OF PABPN1 IN TRANSCRIPTION MAY EXPLAIN ITS HIGH LEVEL OF EXPRESSION IN SE
CELLS[27]. ON THE ONE HAND, IT MAY SEEM COUNTER INTUITIVE THAT A WIDELY EXPRESSED PR
PABPN1 WOULD BE A TARGET ANTIGEN OF CASTRATION-INDUCED IMMUNE RESPONSES IN TH
MODEL. HOWEVER, SAVAGE *ET AL.*[28] DESCRIBED A NATURALLY ARISING CD8+ T CELL RESPONSE AGAI
PEPTIDE DERIVED FROM HISTONE H4, A UBIQUITOUSLY EXPRESSED PROTEIN, IN THE MURINE
PROSTATE CANCER MODEL. MOREOVER, IN OUR STUDY OF TREATMENT-INDUCED AUTOANTI
HUMAN PROSTATE CANCER PATIENTS, MANY OF THE UNDERLYING ANTIGENS WERE WIDELY
[13]. THUS, TREATMENT-INDUCED IMMUNE RESPONSES ARE NOT NECESSARILY DIRECTED AGAI
PROTEINS, BUT MIGHT INSTEAD INVOLVE A BREAKDOWN OF PERIPHERAL TOLERANCE TO WI
PROTEINS, AS OCCURS IN MANY AUTOIMMUNE CONDITIONS.

OUR FINDINGS IN THE SHIONOGI MODEL RAISE CONCERNS ABOUT HUMAN PROSTATE C
TREATMENT-INDUCED AUTOANTIBODY RESPONSES ARE SEEN IN 20-30% OF PATIENTS TREATE
THERAPY AND/OR RADIATION THERAPY. THE RELATIONSHIP BETWEEN TUMOR-ASSOCIATED
CLINICAL OUTCOMES IN HUMANS IS CONTROVERSIAL. AUTOANTIBODIES TO P53 HAVE BEEN
FAVORABLE OUTCOMES IN SOME STUDIES[29, 30] BUT NOT OTHERS[31-35]. AUTOANTIBODIES TO OTHER TARGET
ANTIGENS HAVE BEEN ASSOCIATED WITH IMPROVED PROGNOSIS IN MELANOMA, GLIOBLAST

CANCER AND BREAST CANCER[36-40]. HOWEVER, MOST OF THE ABOVE STUDIES HAVE EXAMINED AUTOAN
PRESENT AT THE TIME OF DIAGNOSIS, WHEREAS OUR WORK IN HUMANS AND THE SHIONOGI M
FOCUSED ON AUTOANTIBODY RESPONSES THAT ARISE DURING TREATMENT. IF THE PRESENT
HUMANS, THEN TREATMENT-INDUCED AUTOANTIBODY AND T CELL RESPONSES MAY PORTEN
THAT SAID IT MAY BE POSSIBLE TO AVERT TUMOR RECURRENCE BY SKEWING THE IMMUNE R
A BENEFICIAL EFFECTOR PHENOTYPE.

REFERENCES

1. HOEPPNER LH, DUBOVSKY JA, DUNPHY EJ, MCNEEL DG. HUMORAL IMMUNE RESPONS TESTIS ANTIGENS IN SERA FROM PATIENTS WITH PROSTATE CANCER. CANCER IMMUN 2006;6:1

2. GARCIA-HERNANDEZ MDE L, GRAY A, HUBBY B, KAST WM. IN VIVO EFFECTS OF VACC WITH SIX-TRANSMEMBRANE EPITHELIAL ANTIGEN OF THE PROSTATE: A CANDIDATE ANTIGEN PROSTATE CANCER. CANCER RES 2007;67:1344-51.

3. EIGL BJ, EGGENER SE, BAYBIK J, ETTINGER S, CHI KN, NELSON C, WANG Z, GLEAVE ME TIMING IS EVERYTHING: PRECLINICAL EVIDENCE SUPPORTING SIMULTANEOUS RATHER THAN CHEMOHORMONAL THERAPY FOR PROSTATE CANCER. CLIN CANCER RES 2005;11:4905-11.

4. RODEN AC, MOSER MT, TRI SD, MERCADER M, KUNTZ SM, DONG H, HURWITZ AA, MCKEAN DJ, CELIS E, LEIBOVICH BC, ALLISON JP, KWON ED. AUGMENTATION OF T CELL LEVE RESPONSES INDUCED BY ANDROGEN DEPRIVATION. J IMMUNOL 2004;173:6098-108.

5. SAVILL J, FADOK V. CORPSE CLEARANCE DEFINES THE MEANING OF CELL DEATH. NA 2000;407:784-8.

6. MERCADER M, BODNER BK, MOSER MT, KWON PS, PARK ES, MANECKE RG, ELLIS TM, WOJCIK EM, YANG D, FLANIGAN RC, WATERS WB, KAST WM, ET AL. T CELL INFILTRATION OF PROSTATE INDUCED BY ANDROGEN WITHDRAWAL IN PATIENTS WITH PROSTATE CANCER. PR USA. 2001;98:14565-70.

7. MATZINGER P. TOLERANCE, DANGER, AND THE EXTENDED FAMILY. ANN REV IMMUN 1994;12:991-1045.

8. MCBRIDE WH, CHIANG CS, OLSON JL, WANG CC, HONG JH, PAJONK F, DOUGHERTY GJ, IWAMOTO KS, PERVAN M, LIAO YP. A SENSE OF DANGER FROM RADIATION. RADIAT RES 2004;1

9. GARNETT CT, PALENA C, CHAKRABORTY M, TSANG KY, SCHLOM J, HODGE JW. SUBLET IRRADIATION OF HUMAN TUMOR CELLS MODULATES PHENOTYPE RESULTING IN ENHANCED T LYMPHOCYTES. CANCER RES 2004;64:7985-94.

10. CHAKRABORTY M, ABRAMS SI, CAMPHAUSEN K, LIU K, SCOTT T, COLEMAN CN, HODG JW. IRRADIATION OF TUMOR CELLS UP-REGULATES FAS AND ENHANCES CTL LYTIC ACTIVITY ADOPTIVE IMMUNOTHERAPY. J IMMUNOL 2003;170:6338-47.

11. FRIEDMAN EJ. IMMUNE MODULATION BY IONIZING RADIATION AND ITS IMPLICATIO CANCER IMMUNOTHERAPY. CURR PHARM DES 2002;8:1765-80.

12. DEMARIA S, BHARDWAJ N, MCBRIDE WH, FORMENTI SC. COMBINING RADIOTHERAPY IMMUNOTHERAPY: A REVIVED PARTNERSHIP. INT J RADIAT ONCOL BIOL PHYS 2005;63:655-66.

13. NESSLINGER NJ, SAHOTA RA, STONE B, JOHNSON K, CHIMA N, KING C, RASMUSSEN D BISHOP D, RENNIE PS, GLEAVE M, BLOOD P, PAI H, ET AL. STANDARD TREATMENTS INDUCE AN SPECIFIC IMMUNE RESPONSES IN PROSTATE CANCER. CLIN CANCER RES 2007;13:1493-502.

14. MIYAKE H, NELSON C, RENNIE PS, GLEAVE ME. TESTOSTERONE-REPRESSED PROSTAT MESSAGE-2 IS AN ANTIAPOPTOTIC GENE INVOLVED IN PROGRESSION TO ANDROGEN INDEPEN CANCER. CANCER RES 2000;60:170-6.

15. MINESITA T, YAMAGUCHI K. AN ANDROGEN-DEPENDENT MOUSE MAMMARY TUMOR RES 1965;25:1168-75.

16. RENNIE PS, BRUCHOVSKY N, AKAKURA K, GOLDENBERG SL, OTAL N, AKAKURA S, W TENNISWOOD M. EFFECT OF TUMOUR PROGRESSION ON THE ANDROGENIC REGULATION OF T RECEPTOR, TRPM-2 AND YPT1 GENES IN THE SHIONOGI CARCINOMA. J STEROID BIOCHEM MOL 1994;50:31-40.

17. BRUCHOVSKY N, RENNIE PS, COLDMAN AJ, GOLDENBERG SL, TO M, LAWSON D. EFFE
ANDROGEN WITHDRAWAL ON THE STEM CELL COMPOSITION OF THE SHIONOGI CARCINOMA. CA
1990;50:2275-82.

18. SKOV K, ADOMAT H, BOWDEN M, DRAGOWSKA W, GLEAVE M, KOCH CJ, WOO J, YAPP
DT. HYPOXIA IN THE ANDROGEN-DEPENDENT SHIONOGI MODEL FOR PROSTATE CANCER AT T
RADIAT RES 2004;162:547-53.

19. SHMUELI O, HORN-SABAN S, CHALIFA-CASPI V, SHMOISH M, OPHIR R, BENJAMIN-ROD
H, SAFRAN M, DOMANY E, LANCET D. GENENOTE: WHOLE GENOME EXPRESSION PROFILES IN
HUMAN TISSUES. C R BIOL 2003;326:1067-72.

20. LIEBERMAN J. THE ABCS OF GRANULE-MEDIATED CYTOTOXICITY: NEW WEAPONS IN
ARSENAL. NAT REV IMMUNOL 2003;3:361-70.

21. CURIEL TJ, COUKOS G, ZOU L, ALVAREZ X, CHENG P, MOTTRAM P, EVDEMON-HOGAN
CONEJO-GARCIA JR, ZHANG L, BUROW M, ZHU Y, WEI S, ET AL. SPECIFIC RECRUITMENT OF REG
CELLS IN OVARIAN CARCINOMA FOSTERS IMMUNE PRIVILEGE AND PREDICTS REDUCED SURV
2004;10:942-9.

22. TORLAKOVIC E, SLIPICEVIC A, ROBINSON C, DECOTEAU JF, ALFSEN GC, VYBERG M,
CHIBBAR R, FLORENES VA. PAX-5 EXPRESSION IN NONHEMATOPOIETIC TISSUES. AM J CLIN PA
2006;126:798-804.

23. DE VISSER KE, EICHTEN A, COUSSENS LM. PARADOXICAL ROLES OF THE IMMUNE SY
DURING CANCER DEVELOPMENT. NAT REV CANCER 2006;6:24-37.

24. MENGSHOL JA, GOLDEN-MASON L, ROSEN HR. MECHANISMS OF DISEASE: HCV-INDUC
LIVER INJURY. NAT CLIN PRACT GASTROENTEROL HEPATOL 2007;4:622-34.

25. LUNDIN BS, ENARSSON K, KINDLUND B, LUNDGREN A, JOHNSSON E, QUIDING-JARBR
SVENNERHOLM AM. THE LOCAL AND SYSTEMIC T-CELL RESPONSE TO HELICOBACTER PYLOR
CANCER PATIENTS IS CHARACTERISED BY PRODUCTION OF INTERLEUKIN-10. CLIN IMMUNOL

26. KUHN U, WAHLE E. STRUCTURE AND FUNCTION OF POLY(A) BINDING PROTEINS. BIO
BIOPHYS ACTA 2004;1678:67-84.

27. HOSODA N, LEJEUNE F, MAQUAT LE. EVIDENCE THAT POLY(A) BINDING PROTEIN C1
NUCLEAR PRE-MRNA POLY(A) TAILS. MOL CELL BIOL 2006;26:3085-97.

28. SAVAGE PA, VOSSELLER K, KANG C, LARIMORE K, RIEDEL E, WOJNOONSKI K, JUNGB
AA, ALLISON JP. RECOGNITION OF A UBIQUITOUS SELF ANTIGEN BY PROSTATE CANCER-INFIL
LYMPHOCYTES. SCIENCE 2008;319:215-20.

29. WINTER SF, SEKIDO Y, MINNA JD, MCINTIRE D, JOHNSON BE, GAZDAR AF, CARBONE
ANTIBODIES AGAINST AUTOLOGOUS TUMOR CELL PROTEINS IN PATIENTS WITH SMALL-CELL
ASSOCIATION WITH IMPROVED SURVIVAL. J NATL CANCER INST 1993;85:2012-8.

30. BERGQUIST M, BRATTSTROM D, LARSSON A, HOLMERTZ J, HESSELIUS P, ROSENBERG
WAGENIUS G, BRODIN O. P53 AUTO-ANTIBODIES IN NON-SMALL CELL LUNG CANCER PATIENT
PREDICT INCREASED LIFE EXPECTANCY AFTER RADIOTHERAPY. ANTICANCER RES. 1998;18:199

31. VOGL FD, FREY M, KREIENBERG R, RUNNEBAUM IB. AUTOIMMUNITY AGAINST P53 PR
INVASIVE CANCER WITH POOR SURVIVAL IN PATIENTS WITH AN OVARIAN MASS. BR J CANCE
43.

32. YAMAZAKI Y, CHIBA I, ISHIKAWA M, SATOH C, NOTANI K, OHIRO Y, TOTSUKA Y, MIZ
S, KITAGAWA Y. SERUM P53 ANTIBODIES AS A PROGNOSTIC INDICATOR IN ORAL SQUAMOUS C
ODONTOLOGY 2008;96:32-7.

33. BERGQVIST AS, BERGQVIST M, BRATTSTROM D, HESSELIUS P, LARSSON A, BRODIN O WÆGENIUS G. SERUM P53 AUTOANTIBODIES AS PROGNOSTIC MARKER IN PATIENTS WITH OESO CARCINOMA. ANTICANCER RES 2001;21:4141-5.

34. GUMUS E, ERDAMAR S, DEMIREL G, HORASANLI K, KENDIRCI M, MIROGLU C. ASSOCI OF POSITIVE SERUM ANTI-P53 ANTIBODIES WITH POOR PROGNOSIS IN BLADDER CANCER PATI 2004;11:1070-7.

35. MACK U, UKENA D, MONTENARH M, SYBRECHT GW. SERUM ANTI-P53 ANTIBODIES IN PATIENTS WITH LUNG CANCER. ONCOL REP 2000;7:669-74.

36. PALLASCH CP, STRUSS AK, MUNNIA A, KONIG J, STEUDEL WI, FISCHER U, MEESE E. AUTOANTIBODIES AGAINST GLEA2 AND PHF3 IN GLIOBLASTOMA: TUMOR-ASSOCIATED AUTO CORRELATED WITH PROLONGED SURVIVAL. INT J CANCER 2005;117:456-9.

37. BACHELOT T, RATEL D, MENETRIER-CAUX C, WION D, BLAY JY, BERGER F. AUTOANT TO ENDOSTATIN IN PATIENTS WITH BREAST CANCER: CORRELATION TO ENDOSTATIN LEVELS BR J CANCER 2006;94:1066-70.

38. GOGAS H, IOANNOVICH J, DAFNI U, STAVROPOULOU-GIOKAS C, FRANGIA K, TSOUTSO PANAGIOTOU P, POLYZOS A, PAPADOPOULOS O, STRATIGOS A, MARKOPOULOS C, BAFALOUKO PROGNOSTIC SIGNIFICANCE OF AUTOIMMUNITY DURING TREATMENT OF MELANOMA WITH I MED 2006;354:709-18.

39. SATZGER I, MEIER A, SCHENCK F, KAPP A, HAUSCHILD A, GUTZMER R. AUTOIMMUNI PROGNOSTIC FACTOR IN MELANOMA PATIENTS TREATED WITH ADJUVANT LOW-DOSE INTERI CANCER 2007;121:2562-6.

40. XUE LJ, SU QS, YANG JH, LIN Y. AUTOIMMUNE RESPONSES INDUCED BY HELICOBACT PYLORI IMPROVE THE PROGNOSIS OF GASTRIC CARCINOMA. MED HYPOTHESES 2008;70:273-6.

FIGURE LEGENDS

Figure 1. RECOGNITION OF PABPN1 BY SERUM ANTIBODIES AND T CELLS FROM CASTRATED M
RECOMBINANT PABPN1 (1 μG) WAS IMMUNOBLOTTED WITH SERUM FROM MICE THAT WERE KNO
POSITIVE (+) OR NEGATIVE (-) FOR AUTOANTIBODIES TO THE ~40 KDA ANTIGEN. THE CORREL
EXPECTED POSITIVES AND NEGATIVES WAS HIGH, ALTHOUGH SOME EXPECTED NEGATIVES W
POSITIVE AGAINST PURIFIED PABPN1, LIKELY REFLECTING THE HIGHER SENSITIVITY OF THIS
THE USE OF RECOMBINANT PABPN1. **B.** SHIONOGI TUMOR LYSATE WAS IMMUNOBLOTTED WIT
NAÏVE DD/S MOUSE AND 5 MICE THAT HAD BEEN IMMUNIZED WITH 100 μG PABPN1 IN INCOMPLETE
FREUND'S ADJUVANT. THE PRESENCE OF AN IMMUNOREACTIVE BAND AT ~40 KDA CONFIRMS
IS THE ~40 KDA ANTIGEN. **C.** WESTERN BLOT SHOWING EXPRESSION OF PABPN1 IN SHIONOGI TUMOR
LYSATE AS WELL AS NORMAL LIVER, LUNG AND UTERINE TISSUES. VARYING AMOUNTS OF P
DEPENDING ON SAMPLE AVAILABILITY AS FOLLOWS: 20 μG SHIONOGI TUMOR LYSATE, 100 μG INTESTINE,
LIVER, MUSCLE, LIVER, 150 μG HEART, LUNG, 33 μG UTERUS. SERUM FROM A PABPN1-IMMUNIZED MOUSE
WAS USED AS PRIMARY ANTIBODY. GAPDH SERVED AS A LOADING CONTROL. **D.** CASTRATIO
PABPN1-SPECIFIC T CELL RESPONSE, AS MEASURED BY IFNγ ELISPOT. FRESH SPLENOCYTES WERE USED
IN ALL ELISPOT EXPERIMENTS. REPRESENTATIVE DATA FROM A SINGLE MOUSE PER TREATM
SHOWN. PABPN1-SPECIFIC T CELLS WERE HIGHEST IN CASTRATED TUMOR-BEARING MICE, FO
CASTRATED NON TUMOR-BEARING MICE. PABPN1-IMMUNIZED MICE SERVED AS A POSITIVE C
NON TUMOR-BEARING, NON-CASTRATED DD/S MICE SERVED AS A NEGATIVE CONTROL. FOR E
SAMPLE WAS RUN IN TRIPLICATE TO PRODUCE AN AVERAGE AND STANDARD DEVIATION.

Figure 2. CASTRATION-INDUCED AUTOANTIBODY AND T CELL RESPONSES TO PABPN1 ARE ASS
TUMOR RECURRENCE. **A.** KAPLAN-MEIER CURVE COMPARING THE TIME TO RECURRENCE OF
WITH (+) OR WITHOUT (-) AUTOANTIBODIES TO PABPN1. **B.** KAPLAN-MEIER CURVE COMPARIN
RECURRENCE OF TUMORS IN MICE WITH (+) OR WITHOUT (-) T CELL RESPONSES TO PABPN1.

Figure 3. RECURRENT SHIONOGI TUMORS RETAIN PABPN1 EXPRESSION. PABPN1 EXPRESSION
EXAMINED IN 15 RECURRENT SHIONOGI TUMORS COMPARED TO ONE PRIMARY TUMOR (1
LOADED WITH 20 μG TUMOR LYSATE AND SCREENED WITH SERUM FROM A PABPN1-IMMUNIZED M
GAPDH SERVED AS A LOADING CONTROL.

Figure 4. CD3+ T CELL INFILTRATION OF SHIONOGI TUMORS AFTER CASTRATION. **A.** ANTI-CD3
SHIONOGI TUMORS SHOWS DENSE INFILTRATION OF CD3+ T CELLS BEGINNING AT DAY 7 POST

REACHING MAXIMAL LEVELS AT DAY 14. BY DAY 28, WHEN MOST OF THE TUMORS HAD RECU
ŒLLS WERE SPARSE (400X). **B.** ANTI-FOXP3 STAINING OF SHIONOGI TUMORS REVEALS THAT A
SUBPOPULATION OF CD3+ T CELLS EXPRESSES FOXP3 (400X). **C.** REPRESENTATIVE RECURRENT
FROM MICE SACRIFICED ON DAY 56 AND DAY 90 POST-CASTRATION. NOTE THAT CD3+ T CELLS
RESTRICTED CONFINED TO THE PERIPHERAL STROMA OF TUMORS (200X).

Hahn et al. Figure 1.

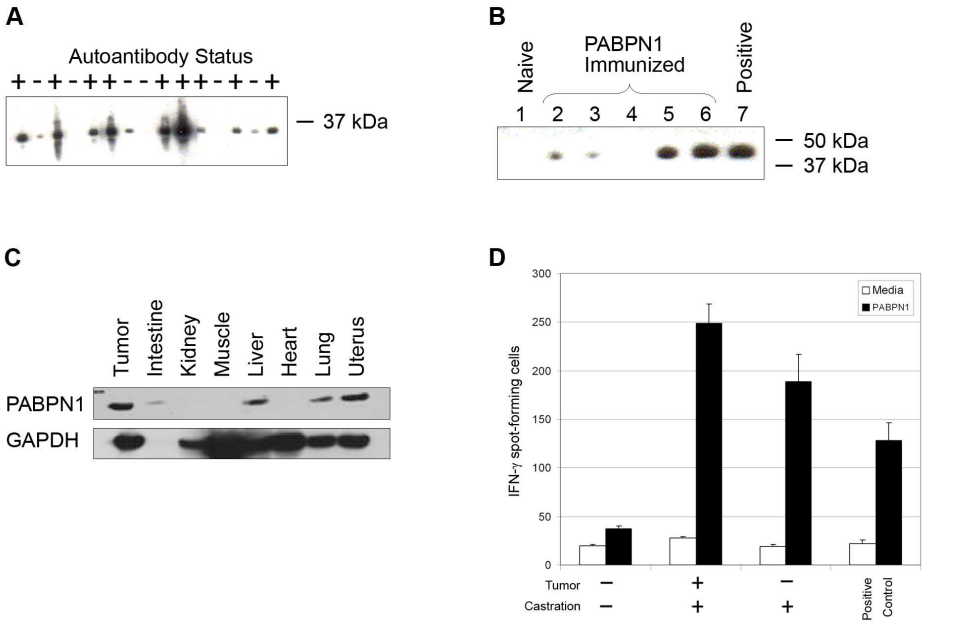


Figure 1
171x127mm (300 x 300 DPI)

Hahn et al. Figure 2.

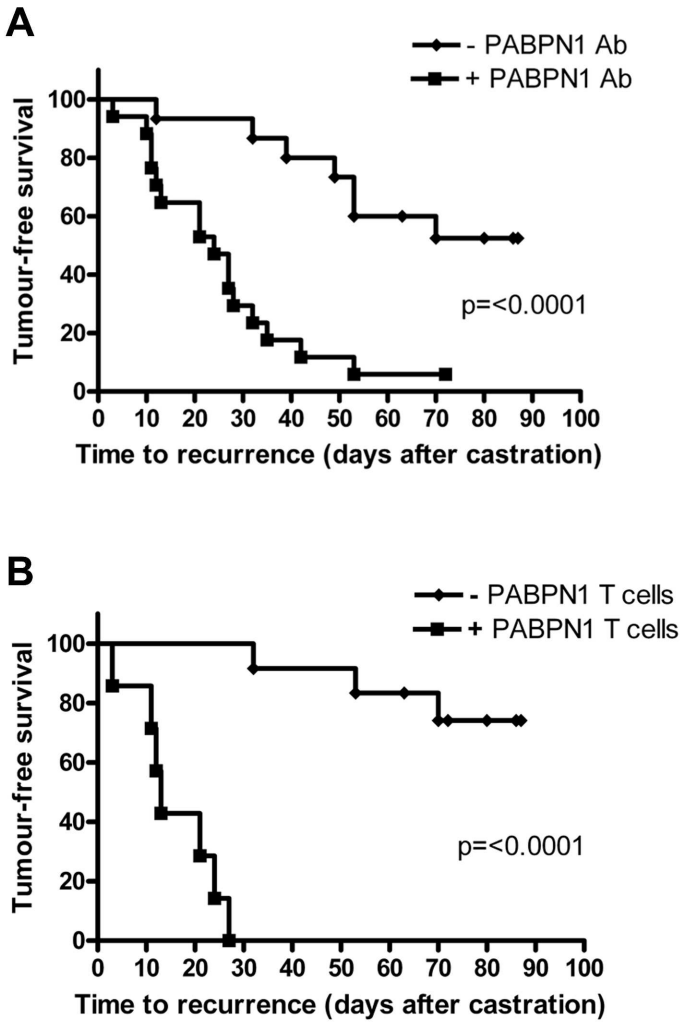


Figure 2
127x211mm (600 x 600 DPI)

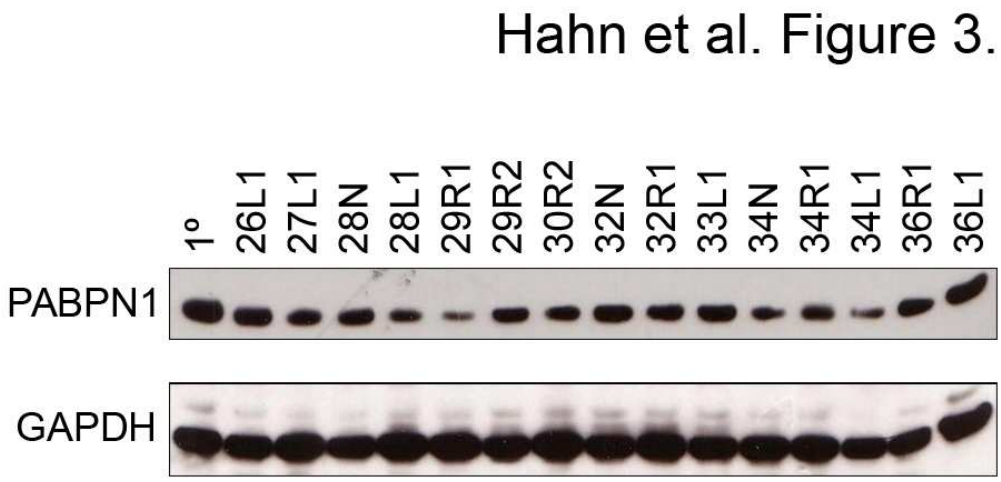


Figure 3
76x43mm (300 x 300 DPI)

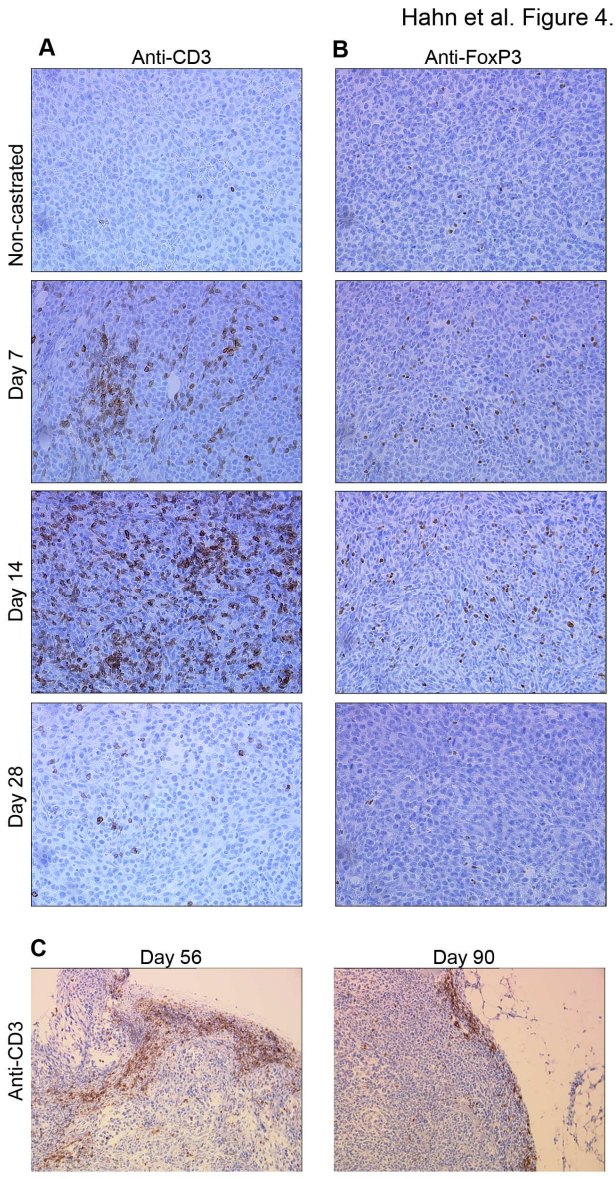


Figure 4
116x215mm (300 x 300 DPI)

SUPPORTING DATA:

Figure 1:

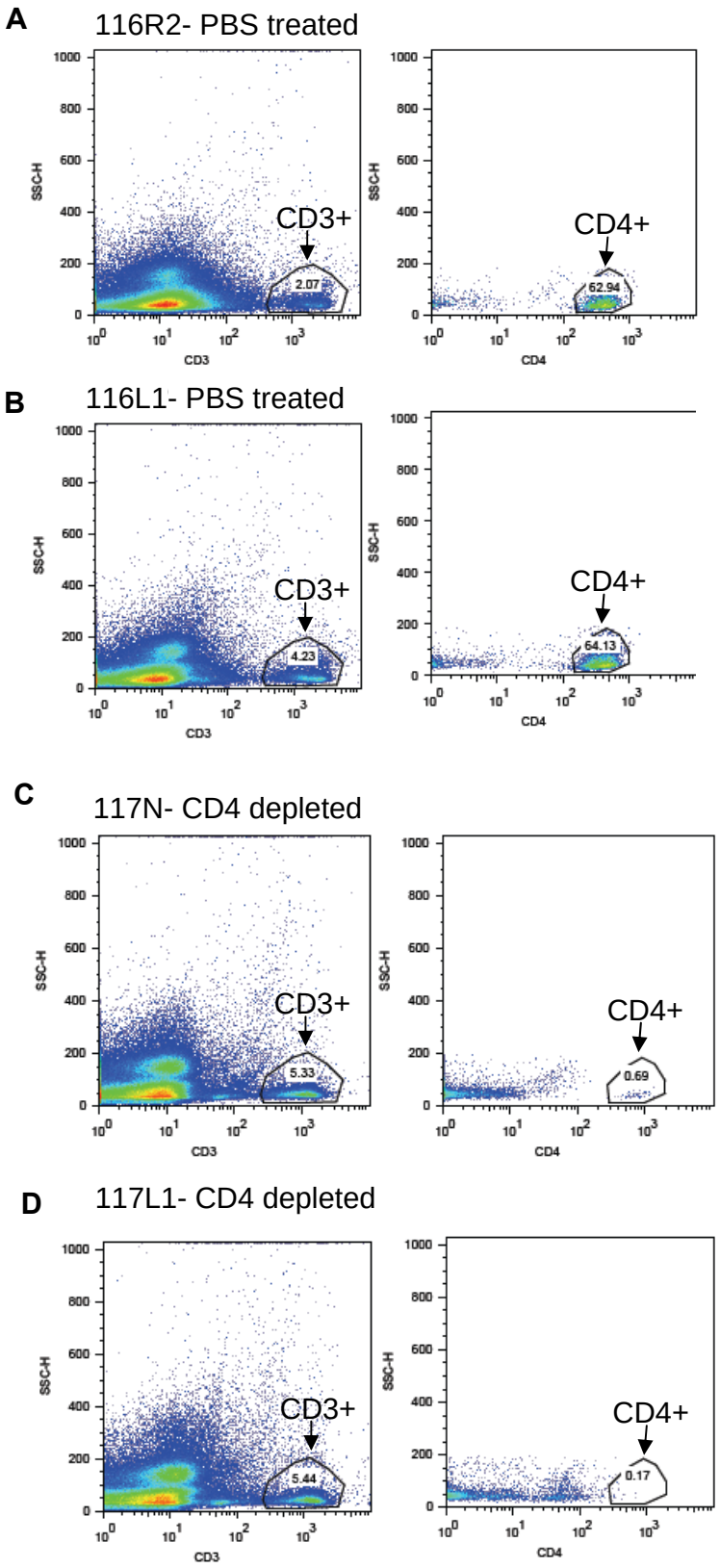


Figure 1:
Flow cytometry data from CD4 depletion experiments. Mice 116R2 (A) and 116L1 (B) were injected with PBS only while 117N (C) and 117L1 (D) were treated with anti-CD4 antibody. PBMCs were isolated from whole blood at Day 66 post-tumor injection. Cells were stained with anti-CD3 and anti-CD4 antibodies for flow cytometric analysis. The first panel shows the total percent of CD3+ T cells and the second panel shows the percent of CD4+ T cells in the CD3+ population. Those mice mice receiving the CD4 depleting antibody have no CD4+ T cells remaining in the CD3+ T cell population whereas the control mice have a healthy CD4+ population, indicating that the depletion was successful.

Figure 2

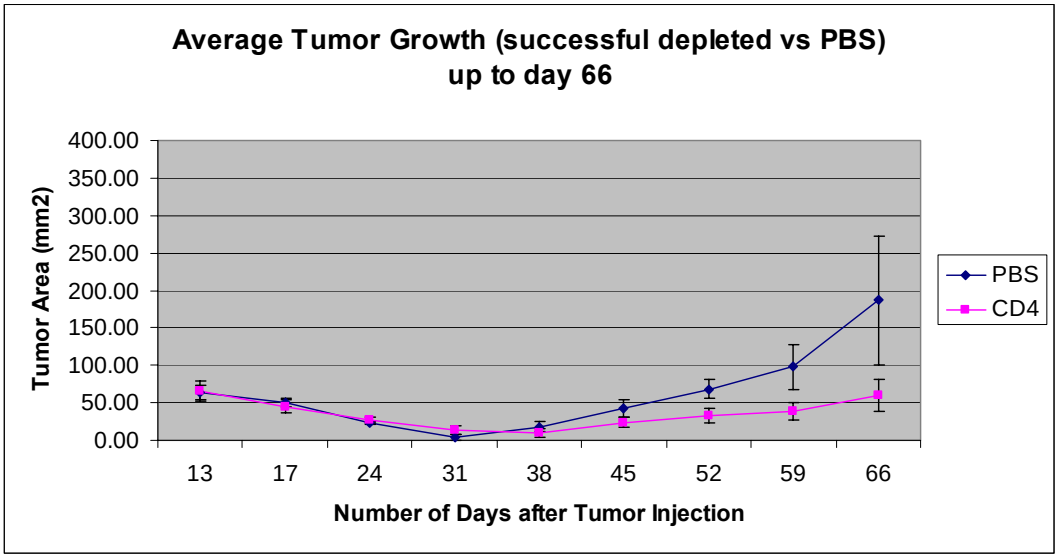


Figure 2: Tumor measurements from the CD4 depletion experiment comparing the average tumor area of the PBS control mice and the CD4 depleted mice. A significant difference between the two groups is demonstrated.

Figure 3

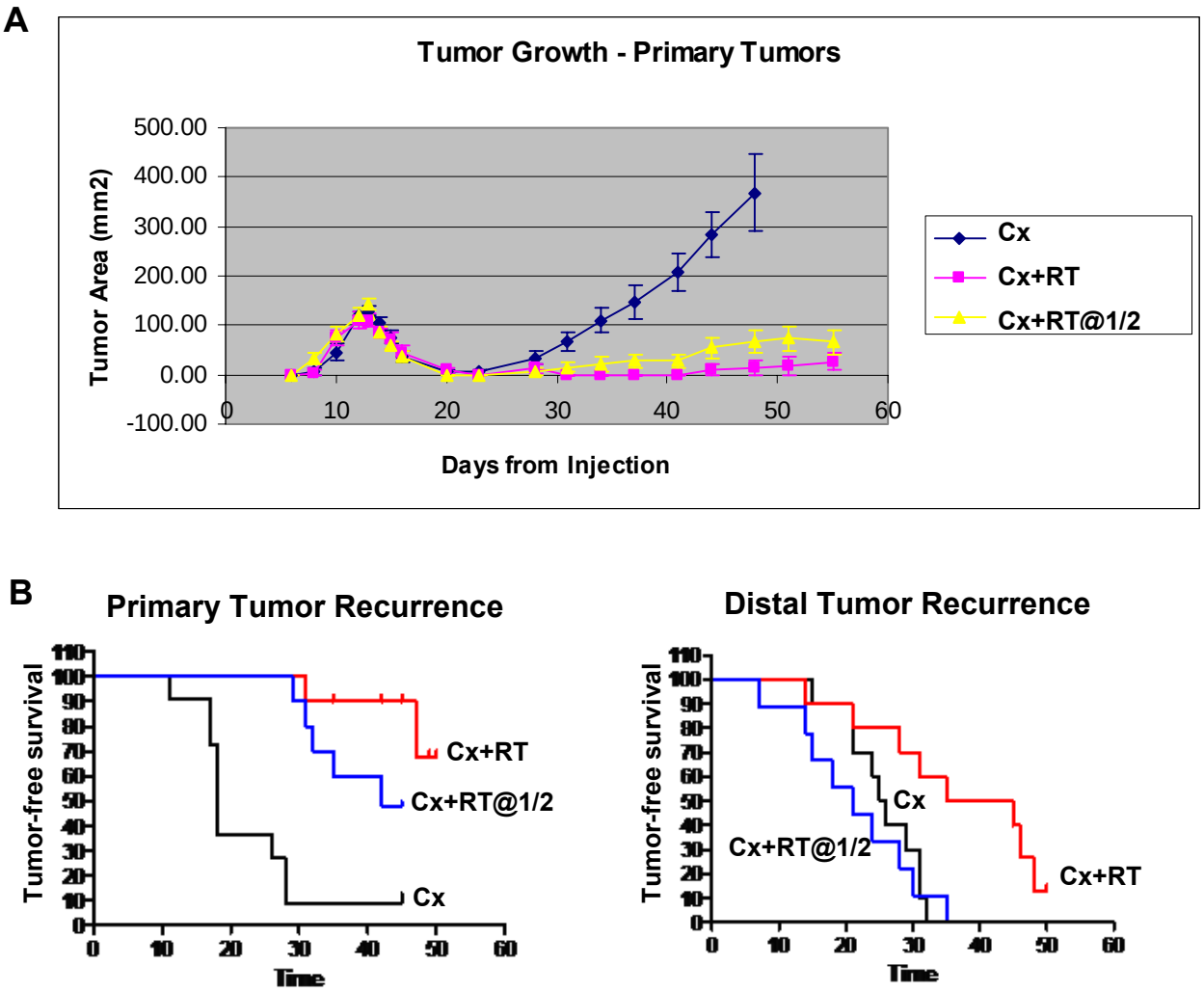
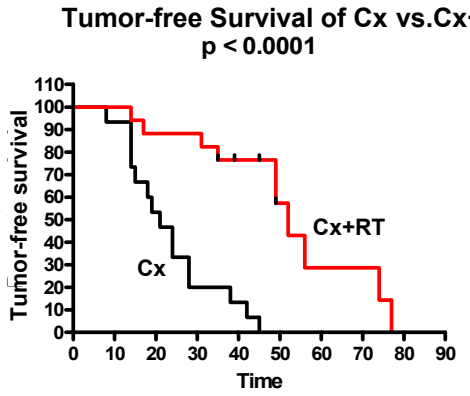
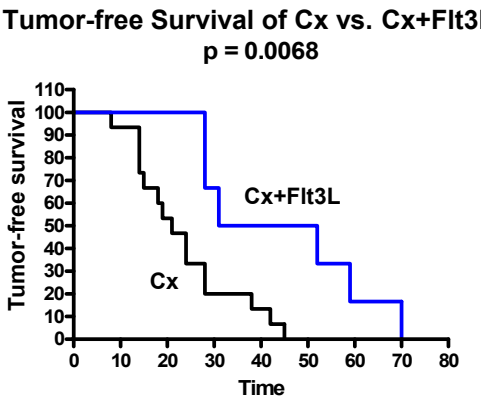
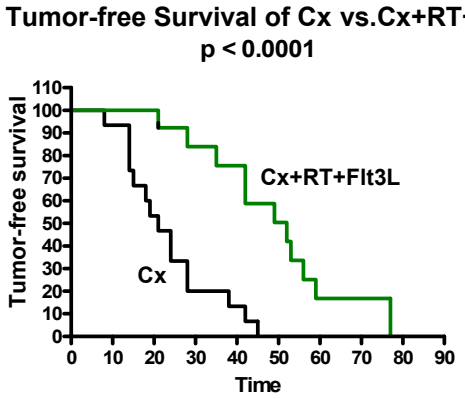


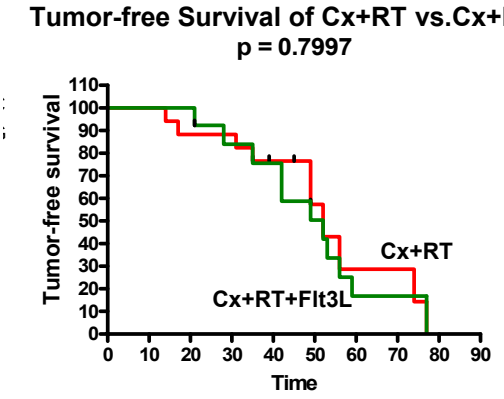
Figure 3: Demonstrating an abscopal response in the Shionogi tumor model. Mice were injected with two Shionogi tumors, one per flank. Tumors were allowed to reach 65-100 mm^2 before all mice were castrated. Some mice received no additional treatment (Cx). A second group of mice received 6 I-125 pellet at the point of maximal tumor regression in the primary tumor only (Cx+RT) while the third group received 6 I-125 pellets when the tumors had only partially regressed (Cx+RT@1/2). Figure 3A shows the rate of tumor growth of the primary tumor and clearly demonstrates the efficacy of radiation on primary tumor growth. Figure 2B shows a Kaplan-Meier curve comparing the rate of tumor recurrence of both the primary and distal tumors. The clear advantage of adding radiation is seen in the primary tumors. In the distal tumors a significant survival advantage is seen in mice treated with Cx+RT, evidence of an abscopal effect. Note this advantage is lost if mice are radiated before the tumors have fully regressed.

Figure 4A


Tumor-free Survival of Cx vs.Cx+RT
p < 0.0001
Tumor-free survival
Time
Cx+RT
Cx


Tumor-free Survival of Cx vs. Cx+Flt3L
p = 0.0068
Tumor-free survival
Time
Cx+Flt3L
Cx


Tumor-free Survival of Cx vs.Cx+RT+Flt3L
p < 0.0001
Tumor-free survival
Time
Cx+RT+Flt3L
Cx


Tumor-free Survival of Cx+RT vs.Cx+RT+Flt3L
p = 0.7997
Tumor-free survival
Time
Cx+RT
Cx+RT+Flt3L

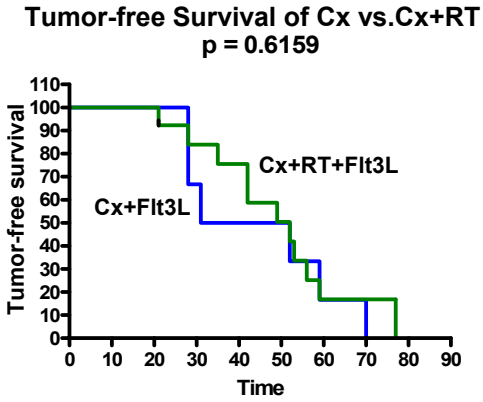

Tumor-free Survival of Cx vs.Cx+RT
p = 0.6159
Tumor-free survival
Time
Cx+RT+Flt3L
Cx+Flt3L

Figure 4B

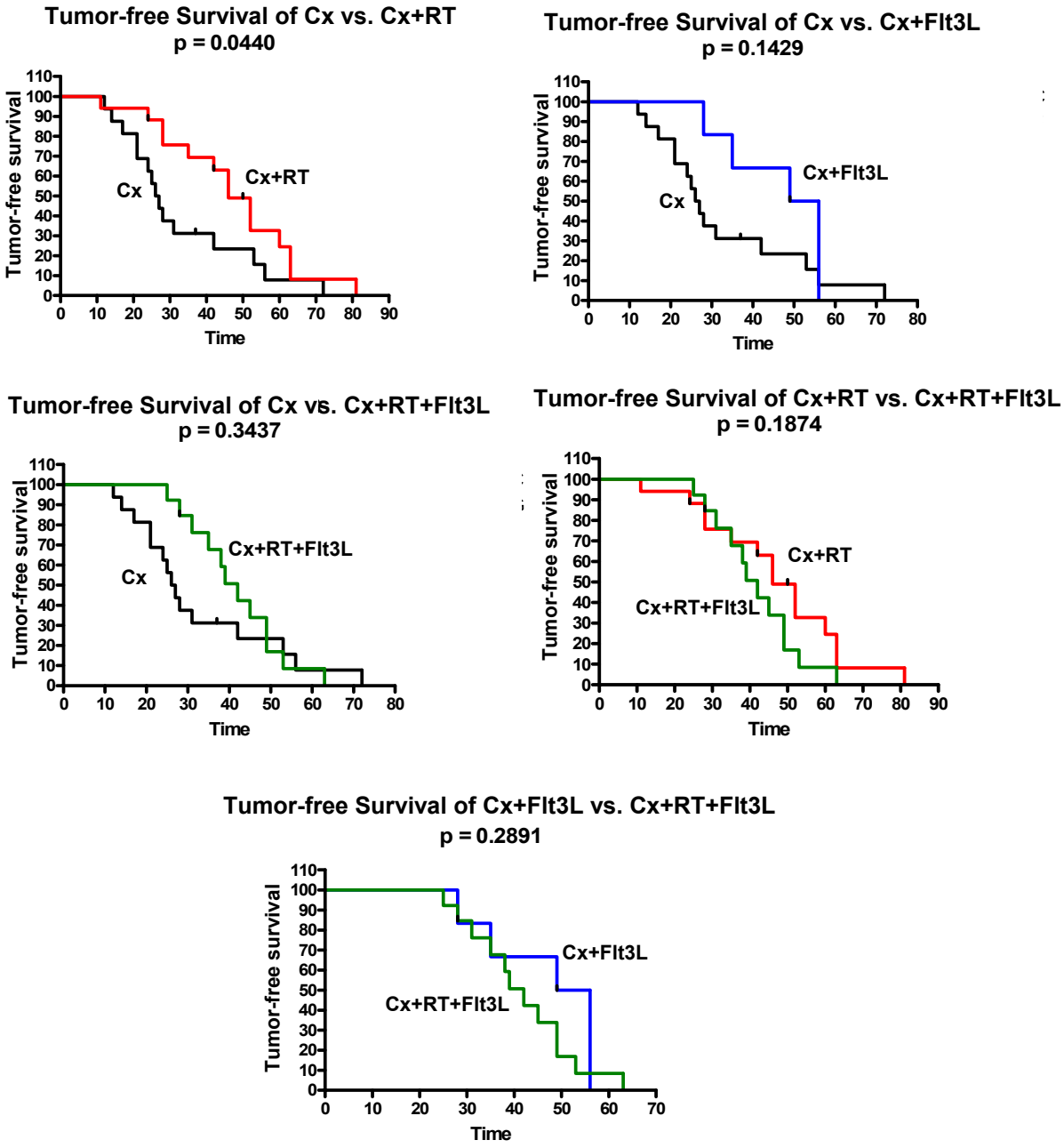


Figure 4: Kaplan-Meier analysis of tumor-free survival in mice treated with a combination of castration, brachytherapy and Flt3 ligand. A survival advantage is seen in the primary tumors of mice treated with a combination of Cx+RT, Cx+Flt3L and Cx+RT+Flt3L compared to Cx alone (Figure 4A). When examining the tumor-free survival of the distal tumors a slight, but significant advantage is seen in those mice treated with Cx+RT, as was noted in Figure 3. However, no additional survival advantage was seen with addition of Flt3L.

Figure 5

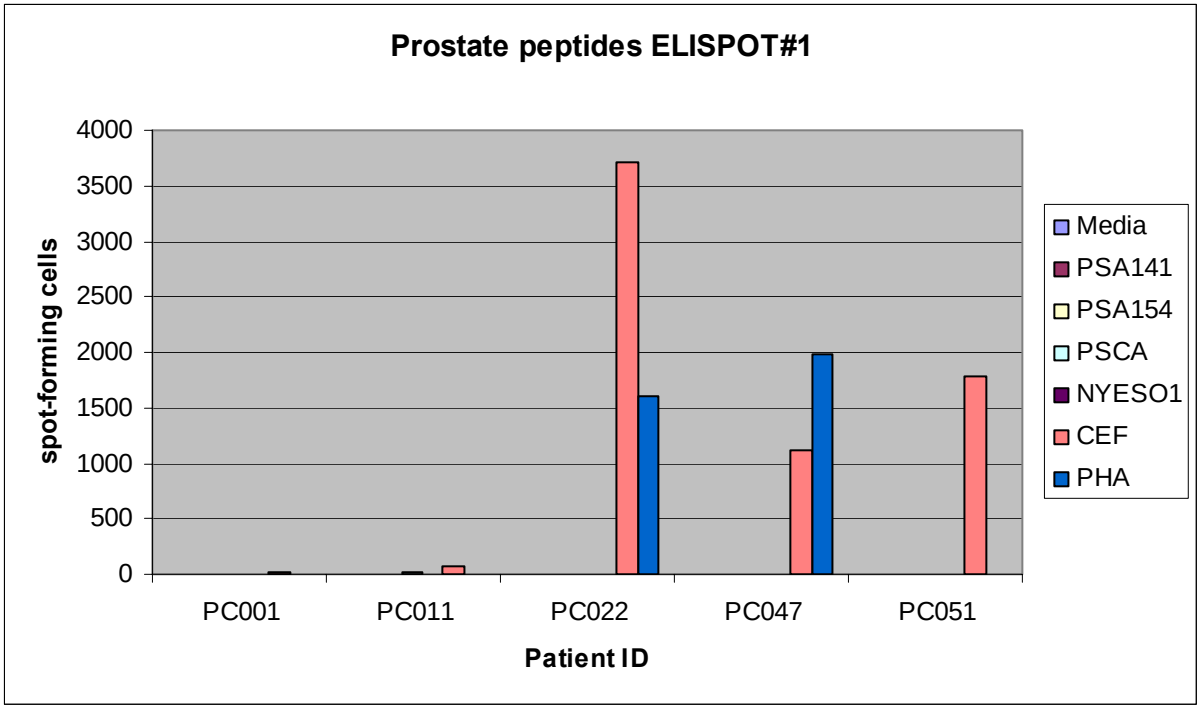


Figure 5: ELISPOT analysis of human prostate cancer patients stimulated with different prostate cancer antigen-specific peptides. Results show that none of the 5 patients tested had antigen-specific T cells against any of the 4 peptides tested. PHA was used as a non-specific T cell stimulant and shows a clear positive result in those 2 patients. In addition, three patients had T cells against the CEF peptide, demonstrating that the ELISPOT methodology worked.